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(54) Title: DETERMINATION OF CANCEROUS CONDITIONS BY MAGE GENE EXPRESSION

(57) Abstract

A method for determining cancers is described. The method involves assaying for expression of a gene coding for at least one of MAGE tumor rejection antigen or its precursor expression product.

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DETERMINATION OF CANCEROUS CONDITIONS BY MAGE GENE EXPRESSION

FIELD OF THE INVENTION

This invention relates to general methods for diagnosing cancers via determining expression of at least one member of the MAGE family of tumor rejection antigen precursors. More particularly, cancers such as lung adenocarcinoma, neck, squamous cell, prostate, and bladder cancers can be diagnosed by determining expression of one or more members of this family of genes. Also a part of the invention are primers which can be used in these methods, such as amplification methods, of which the polymerase chain reaction ("PCR") is the most well known.

BACKGROUND AND PRIOR ART

The study of the recognition or lack of recognition of cancer cells by a host organism has proceeded in many different directions. Understanding of the field presumes some understanding of both basic immunology and oncology.

Early research on mouse tumors revealed that these displayed molecules which led to rejection of tumor cells when transplanted into syngeneic animals. These molecules are "recognized" by T-cells in the recipient animal, and provoke a cytolytic T-cell response with lysis of the transplanted cells. This evidence was first obtained with tumors induced in vitro by chemical carcinogens, such as methylcholanthrene. The antigens expressed by the tumors and which elicited the T-cell response were found to be different for each tumor. See Prehn, et al., J. Natl. Canc. Inst. 18: 769-778 (1957); Klein et al., Cancer Res. 20: 1561-1572 (1960); Gross, Cancer Res. 3: 326-333 (1943), Basombrio, Cancer Res. 30: 2458-2462 (1970) for general teachings on inducing tumors with chemical carcinogens and differences in cell surface antigens. This class of antigens has come to be known as

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"tumor specific transplantation antigens" or "TSTAs". Following the observation of the presentation of such antigens when induced by chemical carcinogens, similar results were obtained when tumors were induced in vitro via ultraviolet radiation. See Kripke, J. Natl. Canc. Inst. 53: 333-1336 (1974).

While T-cell mediated immune responses were observed for the types of tumor described supra, spontaneous tumors were thought to be generally non-immunogenic. These were therefore believed not to present antigens which provoked a response to the tumor in the tumor carrying subject. See Hewitt, et al., Brit. J. Cancer 33: 241-259 (1976).

The family of tum$^-$ antigen presenting cell lines are immunogenic variants obtained by mutagenesis of mouse tumor cells or cell lines, as described by Boon et al., J. Exp. Med. 152: 1184-1193 (1980), the disclosure of which is incorporated by reference. To elaborate, tum$^-$ antigens are obtained by mutating tumor cells which do not generate an immune response in syngeneic mice and will form tumors (i.e., "tum$^+$" cells). When these tum$^+$ cells are mutagenized, they are rejected by syngeneic mice, and fail to form tumors (thus "tum$^-$"). See Boon et al., Proc. Natl. Acad. Sci. USA 74: 272 (1977), the disclosure of which is incorporated by reference. Many tumor types have been shown to exhibit this phenomenon. See, e.g., Frost et al., Cancer Res. 43: 125 (1983).

It appears that tum$^-$ variants fail to form progressive tumors because they initiate an immune rejection process. The evidence in favor of this hypothesis includes the ability of "tum$^-$" variants of tumors, i.e., those which do not normally form tumors, to do so in mice with immune systems suppressed by sublethal irradiation, Van Pel et al., Proc. Natl. Acad. Sci. USA 76: 5282-5285 (1979); and the observation that intraperitoneally injected tum$^-$ cells of mastocytoma P815 multiply exponentially for 12-15 days, and then are eliminated in only a few days in the midst of

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an influx of lymphocytes and macrophages (Uyttenhove et al., J. Exp. Med. 152: 1175-1183 (1980)). Further evidence includes the observation that mice acquire an immune memory which permits them to resist subsequent challenge to the same tum variant, even when immunosuppressive amounts of radiation are administered with the following challenge of cells (Boon et al., Proc. Natl, Acad. Sci. USA 74: 272-275 (1977); Van Pel et al., supra; Uyttenhove et al., supra).

Later research found that when spontaneous tumors were subjected to mutagenesis, immunogenic variants were produced which did generate a response. Indeed, these variants were able to elicit an immune protective response against the original tumor. See Van Pel et al., J. Exp. Med. 157: 1992-2001 (1983). Thus, it has been shown that it is possible to elicit presentation of a so-called "tumor rejection antigen" in a tumor which is a target for a syngeneic rejection response. Similar results have been obtained when foreign genes have been transfected into spontaneous tumors. See Fearon et al., Cancer Res. 48: 2975-1980 (1988) in this regard.

A class of antigens has been recognized which are presented on the surface of tumor cells and are recognized by cytolytic T cells, leading to lysis. This class of antigens will be referred to as "tumor rejection antigens" or "TRAs" hereafter. TRAs may or may not elicit antibody responses. The extent to which these antigens have been studied, has been via cytolytic T cell characterization studies, in vitro i.e., the study of the identification of the antigen by a particular cytolytic T cell ("CTL" hereafter) subset. The subset proliferates upon recognition of the presented tumor rejection antigen, and the cells presenting the antigen are lysed. Characterization studies have identified CTL clones which specifically lyse cells expressing the antigens. Examples of this work may be found in Levy et al., Adv. Cancer Res. 24: 1-59 (1977); Boon et al., J. Exp. Med. 152: 1184-1193 (1980); Brunner et al., J. Immunol. 124: 1627-1634 (1980);

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Maryanski et al., Eur. J. Immunol. 124: 1627-1634 (1980); Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982); Palladino et al., Canc. Res. 47: 5074-5079 (1987). This type of analysis is required for other types of antigens recognized by CTLs, including minor histocompatibility antigens, the male specific H-Y antigens, and the class of antigens referred to as "tum-" antigens, and discussed herein.

A tumor exemplary of the subject matter described supra is known as P815. See DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988); Szikora et al., EMBO J 9: 1041-1050 (1990), and Sibille et al., J. Exp. Med. 172: 35-45 (1990), the disclosures of which are incorporated by reference. The P815 tumor is a mastocytoma, induced in a DBA/2 mouse with methylcholanthrene and cultured as both an in vitro tumor and a cell line. The P815 line has generated many tum$^-$ variants following mutagenesis, including variants referred to as P91A (DePlaen, supra), 35B (Szikora, supra), and P198 (Sibille, supra). In contrast to tumor rejection antigens - and this is a key distinction - the tum$^-$ antigens are only present after the tumor cells are mutagenized. Tumor rejection antigens are present on cells of a given tumor without mutagenesis. Hence, with reference to the literature, a cell line can be tum$^+$, such as the line referred to as "P1", and can be provoked to produce tum$^-$ variants. Since the tum$^-$ phenotype differs from that of the parent cell line, one expects a difference in the DNA of tum$^-$ cell lines as compared to their tum$^+$ parental lines, and this difference can be exploited to locate the gene of interest in tum$^-$ cells. As a result, it was found that genes of tum$^-$ variants such as P91A, 35B and P198 differ from their normal alleles by point mutations in the coding regions of the gene. See Szikora and Sibille, supra, and Lurquin et al., Cell 58: 293-303 (1989). This has proved not to be the case with the TRAs of this invention. These

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papers also demonstrated that peptides derived from the tum antigen are presented by the L^d molecule for recognition by CTLs. P91A is presented by L^d, P35 by D^d and P198 by K^d.

PCT application PCT/US92/04354, filed on May 22, 1992 assigned to the same assignee as the subject application, teaches a family of human tumor rejection antigen precursor coding genes, referred to as the MAGE family, and their expression in various tumor types. Lung adenocarcinoma is not among these. Several of these genes are also discussed in van der Bruggen et al., Science 254: 1643 (1991). It is now clear that the various genes of the MAGE family are expressed in tumor cells, and can serve as markers for the diagnosis of such tumors, as well as for other purposes discussed therein. See also Traversari et al., Immunogenetics 35: 145 (1992); van der Bruggen et al., Science 254: 1643 (1991). The mechanism by which a protein is processed and presented on a cell surface has now been fairly well documented. A cursory review of the development of the field may be found in Barinaga, "Getting Some 'Backbone': How MHC Binds Peptides", Science 257: 880 (1992); also, see Fremont et al., Science 257: 919 (1992); Matsumura et al., Science 257: 927 (1992); Latron et al., Science 257: 964 (1992). These papers generally point to a requirement that the peptide which binds to an MHC/HLA molecule be nine amino acids long (a "nonapeptide"), and to the importance of the first and ninth residues of the nonapeptide.

Studies on the MAGE family of genes have now revealed that, in some cases a nonapeptide is presented on the surface of tumor cells, and that the presentation of the nonapeptide requires that the presenting molecule be HLA-A1. Complexes of the MAGE-1 tumor rejection antigen (the "TRA" or nonapeptide") leads to lysis of the cell presenting it by cytolytic T cells ("CTLs"). Additional research has correlated other nonapeptides derived from MAGE and genes to HLA-A1 and other MHC class I molecules.

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Research presented in, e.g., U.S. patent application Serial No. 07/938,334 filed August 31, 1992, showed that, when comparing homologous regions of various MAGE genes to the region of the MAGE-1 gene coding for the relevant nonapeptide, there is a great deal of homology.

The nucleic acid sequences which code for the nonapeptides were also described therein. These nucleic acid molecules were described as also being useful as diagnostic probes for tumor presence.

The application also showed how it had been found that a cellular model could be used, wherein a non-human cell can be transfected with a nucleic acid sequence coding for a human HLA molecule. The resulting transfectant could then be used to test for nonapeptide specificity of the particular HLA molecule, or as the object of a second transfection with a MAGE gene. The co-transfectant could be used to determine whether the particular MAGE based TRA is presented by the particular HLA molecule.

Many of the references referred to supra present data on the expression pattern of various MAGE genes in different types of cell lines and tumor tissues. What is evident from these data is that there is no "unifying principle" which allows one to predict which MAGE gene will be expressed by a particular tumor type. Thus, while on one level one can say that MAGE genes are "markers" for tumors, on the level of specific tumor types, the correlation of marker and tumor type is not predictable, and must be determined empirically.

It has now been found that one can carry out cancer determination assays by assaying for expression of one or more members of the MAGE family of tumor rejection antigen precursors. How this is accomplished is shown in the examples which follow.

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BRIEF DESCRIPTION OF THE FIGURES

Figures 1A and 1B depict detection of transfectants expressing antigen P815A.

Figure 2 shows the sensitivity of clones P1.HTR, PO.HTR, genomic transfectant P1A.T2 and cosmid transfectant P1A.TC3.1 to lysis by various CTLs, as determined by chromium release assays.

Figure 3 is a restriction map of cosmid C1A.3.1.

Figure 4 shows Northern Blot analysis of expression of gene P1A.

Figure 5 sets forth the structure of gene P1A with its restriction sites.

Figure 6 shows the results obtained when cells were transfected with the gene for P1A, either isolated from P815 or normal cells and then tested with CTL lysis.

Figure 7 shows lytic studies using mast cell line L138. 8A.

Figure 8 is a map of subfragments of the 2.4 kb antigen E fragment which also express the antigen.

Figure 9 shows homology of sections of exon 3 from genes mage 1, 2 and 3.

Figure 10 shows the result of Northern blots for MAGE genes on various tissues.

Figure 11 presents the data of Figure 13 in table form.

Figure 12 shows Southern Blot experiments using the various human melanoma cell lines employed in this application.

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Figure 13 is a generalized schematic of the expression of MAGE 1, 2 and 3 genes by tumor and normal tissues.

Figure 14 shows results from a chromium release assay using CTL clone 20/38 on various cell lines.

Figure 15 presents the result of assays undertaken to determine antigenic specificity of CTL clone 20/38.

Figure 16 shows the results obtained when a TNF release assay was carried out on various transfected cells.

Figure 17 shows results secured from qualitative PCR assays for MAGE-1 in lung adenocarcinomas.

Figure 18 presents data pertaining to quantitative measurement of MAGE-1 expression in lung adenocarcinomas.

Figure 19 shows reverse transcription/PCR amplification production of mRNA extracted from the bladder tumor of a patient referred to as "HM15". This is shown in all lanes marked "R". In lanes marked "D", amplification products of the genomic DNA from the patient are shown.

Figure 20 displays the fraction of tumors expressing genes MAGE-1, 2, 3 and 4 among the superficial and invasive transitional cell carcinomas of the bladder.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Many different "MAGE" genes have been identified, as will be seen from the sequences which follow the application. The protocols described in the following examples were used to isolate these genes and cDNA sequences.

"MAGE" as used herein refers to a nucleic acid sequence isolated from human cells. The acronym "smage" is used to describe sequences of murine origin.

When "TRAP" or "TRAs" are discussed herein as being specific to a tumor type, this means that the molecule under consideration is associated with that type of tumor, although not necessarily to the exclusion of other tumor types.

Example 1

In order to identify and isolate the gene coding for antigen P815A, gene transfection was used. This approach requires both a source of the gene, and a recipient cell line. Highly transfectable cell line P1.HTR was the starting material for the recipient, but it could not be used without further treatment, as it presents "antigen A", one of four recognized P815 tumor antigens. See Van Pel et al., Molecular Genetics 11: 467-475 (1985). Thus, screening experiments were carried out to isolate cell lines which did not express the antigen and which nonetheless possessed P1.HTR's desirable qualities.

To do this, P1.HTR was screened with CTLs which were specific for each of tumor antigens A, B, C and D. Such CTLs are described by Uyttenhove et al., J. Exp. Med. 157: 1040-1052 (1983).

To carry out the selection, 10^6 cells of P1.HTR were mixed with $2-4x10^6$ cells of the CTL clone in a round bottom tube in 2 ml of medium, and centrifuged for three minutes at 150xg. After four hours at 37°C, the cells were washed and resuspended in 10 ml of medium, following Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982). Additional information on the CTL assay and screening protocol, in general may be found in Boon et al., J. Exp. Med. 152: 1184-1193 (1980), and Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982), the disclosures of which are incorporated by reference.

When these selections were carried out, a cell line variant was found which expressed neither antigen A or B. Additional selections with CTLs specific for antigen C then yielded a variant which also lacked antigen C. Please see

figure 2 for a summary of the results of these screenings. The variant PO.HTR is negative for antigens A, B and C, and was therefore chosen for the transfection experiments.

The cell line PO.HTR has been deposited in accordance with the Budapest Treaty at the Institute Pasteur Collection Nationale De Cultures De Microorganismes, 28, Rue de Docteur Roux, 75724 Paris France, and has accession number I-1117.

This methodology is adaptable to secure other cell lines which are variants of a cell type which normally presents at least one of the four recognized P815 tumor antigens, i.e., antigens A, B, C and D, where the variants present none of antigens A, B and C. P1.HTR is a mastocytoma cell line, so it will be seen that the protocol enables the isolation of biologically pure mastocytoma cell lines which express none of P815 antigens A, B and C, but which are highly transfectable. Other tumor types may also be screened in this fashion to secure desired, biologically pure cell lines. The resulting cell lines should be at least as transfectable with foreign DNA as is P1.HTR, and should be selected so as to not express a specific antigen.

Example 2

Previous work reported by DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988) the disclosure of which is incorporated by reference herein had shown the efficacy of using cosmid library transfection to recover genes coding for tum antigens.

Selective plasmid and genomic DNAs of P1.HTR were prepared, following Wölfel et al., Immunogenetics 26: 178-187 (1987). The transfection procedure followed Corsaro et al., Somatic Cell Molec. Genet 7: 603-616 (1981), with some modifications. Briefly, 60 μg of cellular DNA and 3 μg of DNA of plasmid pHMR272, described by Bernard et al., Exp. Cell. Biol. 158: 237-243 (1985) were mixed. This plasmid confers hygromycin resistance upon recipient cells,

and therefore provides a convenient way to screen for transfectants. The mixed DNA was combined with 940 ul of 1 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, and 310 ul 1M $CaCl_2$. The solution was added slowly, and under constant agitation to 1.25 ml of 50 mM Hepes, 280 mM NaCl, 1.5 mM Na_2HPO_4, adjusted to pH 7.1 with NaOH. Calcium phosphate - DNA precipitates were allowed to form for 30-45 minutes at room temperature. Following this, fifteen groups of PO.HTR cells ($5x10^6$ per group) were centrifuged for 10 minutes at 400xg. Supernatants were removed, and pellets were resuspended directly into the medium containing the DNA precipitates. This mixture was incubated for 20 minutes at 37°C, after which it was added to an 80 cm^2 tissue culture flask containing 22.5 ml DMEM, supplemented with 10% fetal calf serum. After 24 hours, medium was replaced. Forty-eight hours after transfection, cells were collected and counted. Transfected cells were selected in mass culture using culture medium supplemented with hygromycin B (350 ug/ml). This treatment selected cells for hygromycin resistance.

For each group, two flasks were prepared, each containing $8x10^6$ cells in 40 ml of medium. In order to estimate the number of transfectants, $1x10^6$ cells from each group were plated in 5 ml DMEM with 10% fetal calf serum (FCS), 0.4% bactoagar, and 300 ug/ml hygromycin B. The colonies were then counted 12 days later. Two independent determinations were carried out and the average taken. This was multiplied by 5 to estimate the number of transfectants in the corresponding group. Correction had to be made for the cloning efficiency of P815 cells, known to be about 0.3.

Example 3

Eight days after transfection as described in example 2, supra, antibiotic resistant transfectants were separated from dead cells, using density centrifugation with Ficoll-

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Paque. These cells were maintained in non-selective medium for 1 or 2 days. The cells were plated in 96 well microplates (round bottom), at 30 cells/microwell in 200 ul of culture medium. Anywhere from 100-400 microwells were prepared, depending on the number of transfectants prepared. Agar colony tests gave estimates of 500-3000. After 5 days, the wells contained about $6x10^4$ cells and replicate plates were prepared by transferring 1/10 of the wells to microplates which were then incubated at 30°C. One day later, master plates were centrifuged, medium removed, and 750 CTLs against P815 antigen A (CTL·P1:5) were added to each well together with 10^6 irradiated syngeneic feeder spleen cells in CTL culture medium containing 40 U/ml recombinant human IL-2, and HAT medium to kill stimulator cells. Six days later, plates were examined visually to identify wells where CTLs had proliferated. Where plates showed proliferating microcultures, aliquots of 100 ul of the wells were transferred to another plate containing ^{51}Cr labeled P1.HTR target cells ($2x10^3$ - $4x10^3$ per well), and chromium release was measured after 4 hours. Replicate microcultures corresponding to those showing high CTL activity were expanded and cloned by limited dilution in DMEM with 10% FCS. Five days later, about 200 clones were collected and screened with the CTL.P1:5 cell line, described supra, in a visual lysis assay. See figure 1A for these results.

In these experiments, three of the fifteen groups of transfectants yielded a few positive microcultures. These microcultures were tested for lytic activity against P1.HTR, as described supra. Most of the microcultures where proliferation was observed showed lytic activity. This activity was well above background, as shown in figure 1B. This figure summarizes data wherein two groups of cells (groups "5" and "14"), 400 and 300 microwells were seeded with 30 hygromycin resistant transfected cells. Amplification and duplication of the microcultures was followed by addition of anti-A CTL P1:5. Six days later,

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lytic activity against P1.HTR was tested. In the figure, each point represents lytic activity of a single microculture.

Duplicate microcultures corresponding to several positive wells were subcloned, and more than 1% of the subclones were found to be lysed by anti-A CTL. Thus, three independent transfectants expressing P815A were obtained from 33,000 hygromycin resistant transfectants. One of these lines, referred to hereafter as P1A.T2 was tested further.

The relevant antigen profile of P1A.T2 is shown in figure 2, this being obtained via anti-CTL assays of the type described supra.

Example 4

The CTL assays carried out for P1A.T2 demonstrated that it presented antigen A ("P815A"), and therefore had received the gene from P1.HTR. To that end, this cell line was used as a source for the gene for the antigen precursor in the following experiments.

Prior work had shown that genes coding for tum antigens could be recovered directly from transfectants obtained with a cosmid library. See DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988). This procedure was followed for recovery of the P815 gene.

Total genomic DNA of P1A.T2 was partially digested with restriction endonuclease Sau 3A1, and fractionated by NaCl density gradient ultracentrifugation to enrich for 35-50 kb DNA fragments, following Grosveld et al., Gene 10: 6715-6732 (1982). These fragments were ligated to cosmid arms of C2RB, described by Bates et al., Gene 26: 137-146 (1983), the disclosure of which is incorporated by reference. These cosmid arms had been obtained by cleavage with SmaI and treatment with calf intestinal phosphatase, followed by digestion with BamHI. Ligated DNA was packaged into lambda phage components, and titrated on E. coli ED 8767, following Grosveld et al., supra. Approximately $9x10^5$

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ampicillin resistant colonies were obtained per microgram of DNA insert.

The cosmid groups were amplified by mixing 30,000 independent cosmids with 2 ml of ED 8767 in 10 mM $MgCl_2$, incubated 20 minutes at 37°C, diluted with 20 ml of Luria Bertani ("LB") medium, followed by incubation for one hour. This suspension was titrated and used to inoculate 1 liter of LB medium in the presence of ampicillin (50 ug/ml). At a bacterial concentration of $2x10^8$ cells/ml (OD_{600}=0.8), a 10 ml aliquot was frozen, and 200 ug/ml chloramphenicol was added to the culture for overnight incubation. Total cosmid DNA was isolated by alkaline lysis procedure, and purified on CsCl gradient.

In these experiments, a library of 650,000 cosmids was prepared. The amplification protocol involved the use of 21 groups of approximately 30,000 cosmids.

Example 5

Using the twenty-one groups of cosmids alluded to supra, (60 ug) and 4 ug of pHMR272, described supra, groups of $5x10^6$ PO.HTR cells were used as transfectant hosts. Transfection was carried out in the same manner as described in the preceding experiments. An average of 3000 transfectants per group were tested for antigen presentation, again using CTL assays as described. One group of cosmids repeatedly yielded positive transfectants, at a frequency of about 1/5,000 drug resistant transfectants. The transfectants, as with P1A.T2, also showed expression of both antigen A and B. The pattern of expression of transfectant P1A.TC3.1 is shown in figure 2.

Example 6

As indicated in Example 5, supra, three independent cosmid transfected cells presenting P815A antigen were isolated. The DNA of these transfectants was isolated and packaged directly with lambda phage extracts, following DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278

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(1988). The resulting product was titrated on E. coli ED 8767 with ampicillin selection, as in Example 5. Similarly, amplification of the cosmids and transfection followed Example 5, again using PO.HTR.

High frequencies of transfection were observed, as described in Table 1, which follows:

Table 1. Transfer of the expression of antigen P815A by cosmids obtained by direct packaging

Transfectant obtained with the cosmid library	No. of cosmids obtained by direct packaging of 0.5 μg of DNA	No. of transfectants expressing P815A / no. of HmB' transfectants
TC3.1	32	87/192
TC3.2	32000	49/384
TC3.3	44	25/72

The cosmids were analyzed with restriction enzymes and it was found that directly packaged transfectant P1A.TC3.1 contained 32 cosmids, 7 of which were different. Each of these 7 cosmids was transfected into PO.HTR, in the manner described supra, and again, following the protocols described above, transfectants were studied for presentation of P815A. Four of the cosmid transfectants showed P815A presentation and, as with all experiments described herein, P815B was co-expressed.

Of the four cosmids showing presentation of the two antigens, cosmid C1A.3.1 was only 16.7 kilobases long, and was selected for further analysis as described infra.

The cosmid C1A.3.1 was subjected to restriction endonuclease analysis, yielding the map shown in Figure 3.

All EcoRI fragments were transfected, again using the above described protocols, and only the 7.4 kilobase fragment produced a transfectant that anti-A CTLs could lyse. Similar experiments were carried out on the PstI

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fragments, and only a 4.1 kb fragment fully contained within the 7.4 kb EcoRI fragment produced lysable transfectants.

This fragment (i.e., the 4.1 kb PstI fragment), was digested with SmaI, giving a 2.3 kb fragment which also yielded host cells presenting antigens A and B after transfection. Finally, a fragment 900 bases long, secured with SmaI/XbaI, also transferred expression of the precursors of these two antigens, i.e., the transfected host cell presented both antigen A and antigen B.

Example 7

The 900 base fragment described above was used as a probe to detect the expression of the P815A gene in parent cell line P1.HTR. To accomplish this, total cellular RNA was first isolated using the guanidine-isothiocyanate procedure of Davis et al., Basic Methods In Molecular Biology (Elsevier Science Publishing Co, New York) (1986). The same reference was the source of the method used to isolate and purify polyA$^+$ mRNA using oligodT cellulose column chromatography.

Samples were then subjected to Northern Blot analysis. RNA samples were fractionated on 1% agarose gels containing 0.66 M formaldehyde. The gels were treated with 10xSSC (SSC: 0.15 M NaCl; 0.015 M sodium citrate, pH 7.0) for 30 minutes before overnight blotting on nitrocellulose membranes. These were baked for two hours at 80°C, after which the membranes were prehybridized for 15 minutes at 60°C in a solution containing 10% dextran sulfate, 1% SDS and 1M NaCl. Hybridization was then carried out using denatured probe (the 900 base fragment), together with 100 ug/ml salmon sperm DNA.

When this protocol was carried out using P1.HTR poly A$^+$ RNA, a band of 1.2 kb and two fainter bands were identified, as shown in Figure 4, lane 1 (6 ug of the RNA).

The same probe was used to screen a cDNA library, prepared from poly-A$^+$ RNA from the cell line. This yielded

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a clone with a 1kb insert, suggesting a missing 5′ end. The Northern blots for the cDNA are not shown.

Hybridization experiments in each case were carried out overnight at 60°C. The blots were washed twice at room temperature with 2xSSC and twice at 60°C with 2xSSC supplemented with 1% SDS.

The foregoing experiments delineated the DNA expressing the P815A antigen precursor sufficiently to allow sequencing, using the well known Sanger dideoxy chain termination method. This was carried out on clones generated using a variety of restriction endonucleases and by specific priming with synthetic oligonucleotide primers. The results for exons of the gene are set forth in SEQUENCE ID NO: 4.

Example 8

The Northern analysis described supra suggested that the 5′ end of the cDNA was missing. To obtain this sequence, cDNA was prepared from P1.HTR RNA using a primer corresponding to positions 320-303. The sequence was then amplified using the polymerase chain reaction using a 3′ primer corresponding to positions 286-266 and a 5′ primer described by Frohman et al., Proc. Natl. Acad. Sci. USA 85: 8998-9002 (1988). A band of the expected size (270 bases) was found, which hybridized to the 900 bp SmaI/XbaI fragment described supra on a Southern blot. Following cloning into m13tg 130 and tg 131, the small, 270 bp fragment was sequenced. The sequence is shown in SEQUENCE ID NO: 1.

Example 9

Following the procurement of the sequences described in Examples 7 and 8 and depicted in SEQ ID NO: 4, a 5.7 kb region of cosmid C1A.3.1 was sequenced. This fragment was known to contain the 900 base fragment which expressed P815A in transfectants. This experiment permitted

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delineation of introns and exons, since the cosmid is genomic in origin.

The delineated structure of the gene is shown in figure 5. Together with SEQ ID NO: 4, these data show that the gene for the antigen precursor, referred to as "P1A" hereafter, is approximately 5 kilobases long and contains 3 exons. An ORF for a protein of 224 amino acids starts in exon 1, ending in exon 2. The 900 base pair fragment which transfers expression of precursors for antigens A and B only contains exon 1. The promoter region contains a CAAT box, as indicated in SEQ ID NO: 1, and an enhancer sequence. This latter feature has been observed in promoters of most MHC class I genes, as observed by Geraghty et al., J. Exp. Med 171: 1-18 (1990); Kimura et al., Cell 44: 261-272 (1986).

A computer homology search was carried out, using program FASTA with K-triple parameters of 3 and 6, as suggested by Lipman et al., Science 227: 1435-1441 (1985), and using Genbank database release 65 (October 1990). No homology was found except for a stretch of 95 bases corresponding to part of an acid region coded by exon 1 (positions 524-618), which is similar to sequences coding for acidic regions in mouse nucleolar protein NO38/B23, as described by Bourbon et al., Mol. Biol. 200: 627-638 (1988), and Schmidt-Zachmann et al., Chromosoma 96: 417-426 (1988). Fifty six of 95 bases were identical. In order to test whether these homologies were the reason for cross hybridizing, experiments were carried out using a mouse spleen cDNA library screened with the 900 base fragment. cDNA clones corresponding closely to the sizes of the cross hybridizing bands were obtained. These were partially sequenced, and the 2.6 kb cDNA was found to correspond exactly to reported cDNA sequence of mouse nucleolin, while the 1.5 kb cDNA corresponded to mouse nucleolar protein NO38/B23.

Analysis of the nucleotide sequence of the gene, referred to as "P1A" hereafter, suggests that its coded

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product has a molecular mass of 25 kd. Analysis of the SEQUENCE ID NO: 4 shows a potential nuclear targeting signal at residues 5-9 (Dingwall et al., Ann. Rev. Cell Biol. 2: 367-390 (1986)), as well as a large acidic domain at positions 83-118. As indicated supra, this contains the region of homology between P1A and the two nucleolar proteins. A putative phosphorylation site can be found at position 125 (serine). Also, a second acidic domain is found close to the C-terminus as an uninterrupted stretch of 14 glutamate residues. A similar C-terminal structure has been found by Kessel et al. Proc. Natl. Acad. Sci. USA 84: 5306-5310 (1987), in a murine homeodomain protein having nuclear localization.

In studies comparing the sequence of gene P1A to the sequences for P91A, P35B and P198, no similarities were found, showing that P1A is indicative of a different class of genes and antigens.

Example 10

With the P1A probe and sequence in hand, investigations were carried out to determine whether the gene present in normal tissue was identical to that expressed by the tumor. To do this, phage libraries were prepared, using lambda zapII 10 and genomic DNA of DBA/2 murine kidney cells. P1A was used as a probe. Hybridization conditions were as described supra, and a hybridizing clone was found. The clone contained exons one and two of the P1A gene, and corresponded to positions -0.7 to 3.8 of figure 5. Following localization of this sequence, PCR amplification was carried out to obtain the sequence corresponding to 3.8 to 4.5 of figure 5.

Sequence analysis was carried out, and no differences were found between the gene from normal kidneys and the P1A gene as obtained from the P815 tumor cells.

In further experiments, the gene as found in DBA/2 kidney cells was transfected into PO.HTR, as described supra. These experiments, presented pictorially in figure

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6, showed that antigens A and B were expressed as efficiently by the kidney gene isolated from normal P815 cells as with the P1A gene isolated from normal kidney cells.

These experiments lead to the conclusion that the gene coding for the tumor rejection antigen precursor is a gene that does not result from a mutation; rather, it would appear that the gene is the same as one present in normal cells, but is not expressed therein. The ramifications of this finding are important, and are discussed infra.

In studies not elaborated upon herein, it was found that variants of the gene were available. Some cells were "P1A^{-}B^{+}", rather than the normal "P1A". The only difference between these is a point mutation in exon 1, with the 18th triplet coding for Ala in the variant instead of Val.

Example 11

Additional experiments were carried out with other cell types. Following the protocols described for Northern blot hybridizations supra, RNA of normal liver and spleen cells was tested to determine if a transcript of the P1A gene could be found. The Northern blot data are presented in figure 4 and, as can be seen, there is no evidence of expression.

The murine P815 cell line from which P1A was isolated is a mastocytoma. Therefore, mast cell lines were studied to determine if they expressed the gene. Mast cell line MC/9, described by Nabel et al., Cell 23: 19-28 (1981), and short term cultures of bone marrow derived mast cells were tested in the manner described supra (Northern blotting), but no transcript was found. In contrast when a BALB/C derived IL-3 dependent cell line L138.8A (Hültner et al., J. Immunol. 142: 3440-3446 (1989)) was tested, a strong signal was found. The mast cell work is shown in figure 4.

It is known that both BALB/C and DBA/2 mice share H-2^{d} haplotype, and thus it was possible to test sensitivity to lysis using the CTLs described supra. Figure 7 shows these

results, which essentially prove that anti-A and anti-B CTLs lysed the cells strongly, whereas anti-C and anti-D lines did not.

Further tests were carried out on other murine tumor cell lines, i.e., teratocarcinoma cell line PCC4 (Boon et al., Proc. Natl. Acad. Sci. USA 74: 272-275 (1977), and leukemias LEC and WEHI-3B. Expression could not be detected in any of these samples.

Example 12

The actual presentation of the P1A antigen by MHC molecules was of interest. To test this, cosmid C1A.3.1 was transfected into fibroblast cell line DAP, which shows phenotype H-2^k. The cell lines were transfected with genes expressing one of the K^d, D^d, and L^d antigen. Following transfection with both the cosmid and the MHC gene, lysis with CTLs was studied, again as described supra. These studies, summarized in Table 2, show that L^d is required for presentation of the P1A antigens A and B.

Table 2. H-2-restriction of antigens P815A and P815B

Recipient cell*	No. of clones lysed by the CTL/no. of HmB' clones*	
	CTL anti-A	CTL anti-B
DAP (H-2^k)	0/208	0/194
DAP + K^d	0/165	0/162
DAP + D^d	0/157	0/129
DAP + L^d	25/33	15/20

*Cosmid C1A.3.1 containing the entire P1A gene was transfected in DAP cells previously transfected with H-2^d class I genes as indicated.

*Independent drug-resistant colonies were tested by lysis by anti-A or anti-B CTL in a visual assay.

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The observation that one may associate presentation of a tumor rejection antigen with a particular MHC molecule was confirmed in experiments with human cells and HLA molecules, as elaborated upon infra.

Example 13

Using the sequence of the P1A gene as well as the amino acid sequence derivable therefrom, antigenic peptides which were A^+ B^+ (i.e., characteristic of cells which express both the A and B antigens), and those which are A^-B^+ were identified. The peptide is presented in SEQ ID NO: 26. This peptide when administered to samples of PO.HTR cells in the presence of CTL cell lines specific to cells presenting it, led to lysis of the PO.HTR cells, lending support to the view that peptides based on the product expressed by the gene can be used as vaccines.

Example 14

The human melanoma cell line referred to hereafter as MZ2-MEL is not a clonal cell line. It expresses four stable antigens recognized by autologous CTLs, known as antigens "D, E, F, and A". In addition, two other antigens "B" and "C" are expressed by some sublines of the tumor. CTL clones specific for these six antigens are described by Van den Eynde et al., Int. J. Canc. 44: 634-640 (1989). Among the recognized subclones of MZ2-MEL are MEL.43, MEL3.0 and MEL3.1. (Van den Eynde et al., supra). Cell line MEL3.1 expresses antigen E, as determined by CTL studies as described for P815 variants, supra, so it was chosen as a source for the nucleic acid sequence expressing the antigen precursor.

In isolating the pertinent nucleic acid molecule for a tumor rejection antigen precursor, the techniques developed supra, showed that a recipient cell is needed which fulfills two criteria: (i) the recipient cell must not express the TRAP of interest under normal conditions,

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and (ii) it must express the relevant class I HLA molecule. Also, the recipient cell must have a high transfection frequency, i.e., it must be a "good" recipient.

In order to secure such a cell line, the clonal subline MEL3.1 was subjected to repeated selection with anti-E CTL 82/30 as described by Van den Eynde, supra. The repeated cycles of selection led to isolation of subclone MZ2-MEL-2.2 isc E^-. This subclone is also $HPRT^-$, (i.e., sensitive to HAT medium: 10^{-4} M hypoxanthine, 3.8 x 10^{-7} aminopterine, 1.6 x 10^{-5} M 2-deoxythymidine). The subclone is referred to as "MEL-2.2" for simplicity hereafter.

Example 15

The genomic DNA of MEL3.0 was prepared following Wölfel et al., Immunogenetics 26: 178-187 (1987), the disclosure of which is incorporated by reference. The plasmid pSVtkneoß, as described by Nicolas et al., Cold Spring Harb., Conf. Cell Prolif. 10: 469-485 (1983) confers geneticin resistance, so it can be used as a marker for cotransfection, as it was in this experiment.

Following a procedure similar but not identical to that of Corsaro et al., Somatic Cell Molec. Genet 7: 603-616 (1981), total genomic DNA and the plasmid were cotransfected. The genomic DNA (60 µg) and plasmid DNA (6 µg) were mixed in 940 µl of 1 mM Tris·HCl (pH 7.5), 0.1 mM EDTA, after which 310 µl of 1M $CaCl_2$ was added. This solution was slowly added, under constant agitation, to 1.25 ml of 2xHBS (50 mM HEPES, 280 mM NaCl 1.5 mM Na_2HPO_4, adjusted to pH 7.1 with NaOH). The calcium phosphate DNA precipitates were allowed to form for 30-45 minutes at room temperature, after which they were applied to 80 cm^2 tissue culture flasks which had been seeded 24 hours previously with $3x10^6$ MEL2.2 cells, in 22.5 ml of melanoma culture medium (Dulbecco's Modified Eagle's Medium) supplemented with 10% fetal calf serum. After 24 hours, the medium was replaced. Forty eight hours after transfection, the cells

were harvested and seeded at $4x10^6$ cells per 80 cm^2 flask in melanoma culture medium supplemented with 2 mg/ml of geneticin. The geneticin serves as a selection marker.

Example 16

Thirteen days after transfection, geneticin-resistant colonies were counted, harvested, and cultured in nonselective medium for 2 or 3 days. Transfected cells were then plated in 96-well microplates at 200 cells/well in 200 ul of culture medium with 20% fetal calf serum (FCS) in order to obtain approximately 30 growing colonies per well. The number of microcultures was aimed at achieving redundancy, i.e., such that every independent transfectant should be represented at least four times.

After 10 days, wells contained approximately $6x10^4$ cells. These cells were detached, and 1/3 of each microculture was transferred to a duplicate plate. After 6 hours, i.e., after readherence, medium was removed and 1500 anti-E CTL (CTL 82/30), were added to each well in 100 μl of CTL culture medium with 35 U/ml of IL-2. One day later, the supernatant (50 μl) was harvested and examined for TNF concentration, for reasons set forth in the following example.

Example 17

The size of the mammalian genome is $6x10^6$ kb. As the average amount of DNA integrated in each drug-resistant transfectant was expected to be about 200 kb, a minimum of 30,000 transfectants would need to be examined to ascertain whether antigen E had been transfected. Prior work with murine cells had shown that when a CTL stimulation assay was used, groups containing only 3% of cells expressing the antigen of interested could be identified. This should reduce the number of assays by a factor of 30. While an anti-E CTL assay, as described supra, in mixed E^+/E^- cells was helpful, it was not sufficient in that consistent results could not be obtained.

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As a result, an alternative test was devised. Stimulation of CTLs was studied by release of tumor necrosis factor ("TNF") using well known methodologies which need not be repeated here. As described in Example 15, 1500 CTL 82/30 cells had been added per well of transfectants. These CTLs were collected 6 days after stimulation. As indicated supra, after 1/3 of the cells in each well had been removed and the remaining 2/3 ($4x10^4$) had readhered, the CTLs and IL-2 were added thereto. The 50 μl of supernatant was removed 24 hours later and transferred to a microplate containing $3x10^4$ W13 (WEHI-164 clone 13; Espevik et al., J. Immunol. Meth. 95: 99-105 (1986)) cells in 50 μl of W13 culture medium (RPMI-1640, supplemented with L-arginine (116 mg/l), L-asparagine (36 mg/l), L-glutamine (216 mg/l), and 10% FCS supplemented with 2 μg of actinomycin D at 37°C in an 8% CO_2 atmosphere. The cell line W13 is a mouse fibrosarcoma cell line sensitive to TNF. Dilutions of recombinant TNF-ß in RPMI 1640 were added to target cell controls.

The W13 cultures were evaluated after 20 hours of incubation, and dead cell percentage was measured using an adaptation of the colorimetric assay of Hansen et al., J. Immunol. Meth. 119: 203-210 (1989). This involved adding 50 ml of (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide at 2.5 mg/ml in PBS, followed by two hours of incubation at 37°C. Dark blue formazan crystals were dissolved by adding 100 μl of lysis solution (1 volume N,N dimethyl formamide mixed at 37°C with two volumes of water containing 30% (w/v) sodium dodecyl sulphate, at pH 4.7 from 1.6% acetic acid and 2.5% 1N HCl). Plates were incubated at 37°C overnight, and ODs were taken at 570 nm using 650 nm as control. Dead cell percentage was determined via the formula:

$$100 \times \left(1 - \frac{100-(OD_{570}\text{ sample well})}{OD_{570}\text{ well + medium}}\right)$$

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following Espevik et al., J. Immunol. Meth. 95: 99-105 (1986). The results showed that even when the ratio of E^+/E^- cells was as low as 1/45, significant production of TNF was observed, thus showing active CTLs. This led to the decision to test the drug resistant transfectants in groups of 30.

Example 18

Cells were tested for TNF production as discussed in Example 17, supra. A total of 100 groups of E^- cells ($4x10^6$ cells/group) were tested following transfection, and $7x10^4$ independent geneticin resistant transfectants were obtained, for an average of 700 per group. Only one group of transfected cells led to a microculture which caused anti-E antigen CTL clone 82/30 to produce TNF. Of 300 clones tested, 8 were positive. These clones were then tested for lysis by anti-E CTL, using the standard ^{51}Cr release assay, and were found to be lysed as efficiently as the original E^+ cell line. The transfectant E.T1, discussed herein, had the same lysis pattern as did MEL2.2 for CTLs against antigens B,C,D and F.

The fact that only one transfectant presented the antigen out of 70,000 geneticin resistance transfectants may at first seem very low, but it is not. The work described supra for P815 showed an average frequency of 1/13,000. Human DNA recipient MEL2.2 appears to integrate 5 times less DNA than P1.HTR.

Example 19

Once transfectant E.T1 was found, analysis had to address several questions including whether an E^+ contaminant of the cell population was the cause. The analysis of antigen presentation, described supra, shows that E.T1 is B^- and C^-, just like the recipient cell MEL2.2. It was also found to be $HPRT^-$, using standard selection

procedures. All E^+ cells used in the work described herein, however, were $HPRT^+$.

It was also possible that an E^+ revertant of MEL2.2 was the source for E.T1. To test this, the observation by Perucho et al., Cell 22: 309-317 (1980), that cotransfected sequences usually integrate together at a single location of recipient genome was employed. If antigen E in a transfectant results from cotransfec-tion with pSVtkneoß, then sequences should be linked and deletion of the antigen might also delete the neighboring pSVtkneoß sequences. Wölfel et al., supra, has shown this to be true. If a normally E^- cell is transfected with pSVtkneoß, then sequences should be linked and deletion of the antigen might also delete the neighboring pSVtkneoß sequences. If a normally E^+ cell transfected with pSVtkneoß is E.T1, however, "co-deletion" should not take place. To test this, the transfectant E.T1 was subjected to immunoselection with 82/30, as described supra. Two antigen loss variants were obtained, which resisted lysis by this CTL. Neither of these had lost geneticin resistance; however, Southern blot analysis showed loss of several neo^r sequences in the variants, showing close linkage between the E gene and neo^r gene in E.T1, leading to the conclusion that E.T1 was a transfectant.

Example 20

The E^+ subclone MZ2-MEL 43 was used as a source of DNA for preparation of a cosmid library. This library of nearly 700,000 cosmids was transfected into MZ2-MEL 2.2 cells, following the cosmid transfection protocols described supra.

By packaging the DNA of cosmid transfectants directly into lambda phage components, it is sometimes possible to retrieve cosmids that contain the sequences of interest. This procedure was unsuccessful here, so rescue of the transfected sequence was accomplished by ligating DNA of

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the transfectant to appropriate restriction fragments of cosmid vector pTL6. This was tried with two transfectants and was successful with one of them. One cosmid, referred to as B3, was recovered from this experiment, and subjected to restriction endonuclease digestion via XmaI, or by BamHI digestion of a large, 12 kb XmaI transfected fragment. The fragments were cloned into vector pTZ 18R, and then transfected into MEL2.2. Again, TNF production was the measure by which successful transfection was determined. The experiments led to the determination of a gene sequence capable of transfecting antigen E on the 12 kb XmaI fragment, and then on the 2.4 kb fragment of BamHI digestion of the 12 kb segment.

The 2.4 kb fragment hybridizes with a 2.4 kb fragment from MZ2-MEL and with a T cell clone of patient MZ-2, as determined by Southern Blots (BamHI digested DNA). The band is absent from E⁻ antigen loss variants of MZ2-MEL, as seen in SEQ ID NO: 7.

The sequence for the E antigen precursor gene has been determined, and is presented herein:

Example 21

After the 2.4 kb genomic segment had been identified, studies were carried out to determine if an "E^+" subline expressed any homologous DNA. Cell line MZ2-MEL 3.0 was used as a source, and a cDNA library was prepared from its mRNA, using art known techniques. The 2.4 kb segment was used as a probe, and an mRNA of about 1.8 kb was identified as homologous, using Northern blot analysis. When cDNA was screened, clones were obtained showing almost complete identity to parts of the 2.4 kb fragment. Two exons were thus identified. An additional exon was located upstream of these, via sequencing segments of cosmid B3 located in front of the 2.4 kb BamHI fragment. The gene extends over about 4.5 kb, as shown in Figure 8. The starting point of the transcribed region was confirmed using PCR for the 5′ end of the cDNA. The three exons comprise 65, 73, and 1551

base pairs. An ATG is located at position 66 of exon 3, followed by a 927 base pair reading frame.

Example 22

To determine if smaller segments of the 2.4 kb fragment could transfer the expression of antigen E, smaller pieces corresponding to the larger gene were prepared, using art recognized techniques, and transferred into E⁻ cells. Figure 8 shows the boundaries of the three segments.

Transfer of antigen expression in this manner indicates that the gene codes for the antigen precursor, rather than coding for a protein which activates the antigen.

Example 23

The probing of cDNA described supra revealed, surprisingly, two different but closely related cDNAs. These cDNAs, when tested, did not transfer expression of antigen E, but they do show substantial homology to the first cDNA segment. The three segments, appear to indicate a newly recognized family of genes, referred to as "MAGE" for "melanoma antigen". In Figure 9, MAGE-1" directs expression of the antigen from MZ2 cells. Portions of the third exon of each gene are presented in Figure 9. The second and third sequences are more closely related to each other than the first (18.1 and 18.9% difference compared to the first; 12% with each other). Out of 9 cDNA clones obtained, three of each type were obtained, suggesting equal expression. "MAGE" as used hereafter refers to a family of molecules, and the nucleic acids coding for them. These nucleic acids share a certain degree of homology and are expressed in tumor cells including several types of human tumor cells as well as in human tumors. The family is referred to as "MAGE" because the first members were identified in human melanoma cells. As the experiments which follow indicate, however, the members of the MAGE

family are not at all restricted to melanoma tumors; rather, MAGE refers to a family of tumor rejection antigen precursors and the nucleic acid sequences coding therefore. The antigens resulting therefrom are referred to herein as "MAGE TRAs" or "melanoma antigen tumor rejection antigens"

Example 24

Experiments with mouse tumors have demonstrated that new antigens recognized by T cells can result from point mutations that modify active genes in a region that codes for the new antigenic peptide. New antigens can also arise from the activation of genes that are not expressed in most normal cells. To clarify this issue for antigen MZ2-E, the MAGE-1 gene present in the melanoma cells was compared to that present in normal cells of patient MZ2. Amplification by polymerase chain reaction (PCR) of DNA of phytohemagglutinin-activated blood lymphocytes using primers surrounding a 1300 bp stretch covering the first half of the 2.4 kb fragment was carried out. As expected, a PCR product was obtained whereas none was obtained with the DNA of the E^- variant. The sequence of this PCR product proved identical to the corresponding sequence of the gene carried by the E^+ melanoma cells. Moreover, it was found that antigen MZ2-E was expressed by cells transfected with the cloned PCR product. This result suggests that the activation of a gene normally silent is responsible for the appearance of tumor rejection antigen MZ2-E.

Example 25

In order to evaluate the expression of gene MAGE-1 by various normal and tumor cells, Northern blots were hybridized with a probe covering most of the third exon. In contrast with the result observed with human tumor cell line MZ2-MEL 3.0, no band was observed with RNA isolated from a CTL clone of patient MZ2 and phytohemagglutinin-activated blood lymphocytes of the same patient. Also negative were several normal tissues of other individuals

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(Figure 10 and Figure 11). Fourteen melanoma cell lines of other patients were tested. Eleven were positive with bands of varying intensities. In addition to these cultured cell lines, four samples of melanoma tumor tissue were analyzed. Two samples, including a metastasis of patient MZ2, proved positive, excluding the possibility that expression of the gene represented a tissue culture artefact. A few tumors of other histological types, including lung tumors were tested. Most of these tumors were positive (Figures 10 and 11). These results indicated that the MAGE gene family is expressed by many melanomas and also by other tumors. However, they provided no clear indication as to which of genes MAGE-1, 2 or 3 were expressed by these cells, because the DNA probes corresponding to the three genes cross-hybridized to a considerable extent. To render this analysis more specific, PCR amplification and hybridization with highly specific oligonucleotide probes were used. cDNAs were obtained and amplified by PCR using oligonucleotide primers corresponding to sequences of exon 3 that were identical for the three MAGE genes discussed herein. The PCR products were then tested for their ability to hybridize to three other oligonucleotides that showed complete specificity for one of the three genes (Figure 9). Control experiments carried out by diluting RNA of melanoma MZ2-MEL 3.0 in RNA from negative cells indicated that under the conditions used herein the intensity of the signal decreased proportionally to the dilution and that positive signals could still be detected at a dilution of 1/300. The normal cells (lymphocytes) that were tested by PCR were confirmed to be negative for the expression of the three MAGE genes, suggesting therefore a level of expression of less than $1/300^{th}$ that of the MZ2 melanoma cell line (Figure 11). For the panel of melanoma cell lines, the results clearly showed that some melanomas expressed MAGE genes mage 1, 2 and 3 whereas other expressed only mage-2 and 3 (Figures 11 and 10). Some of the other tumors also

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expressed all three genes whereas others expressed only MAGE-2 and 3 or only MAGE-3. It is impossible to exclud formally that some positive PCR results do not reflect the expression of one of the three characterized MAGE genes but that of yet another closely related gene that would share the sequence of the priming and hybridizing oligonucleotides. It can be concluded that the MAGE gene family is expressed by a large array of different tumors and that these genes are silent in the normal cells tested to this point.

Example 26

The availability of a sequence that transfects at high efficiency and efficiently expresses a TRAP made it possible to search for the associated major histocompatibility complex (MHC) class I molecule. The class I specificities of patient MZ2 are HLA-A1, A29, B37, B44 and C6. Four other melanomas of patients that had A1 in common with MZ2 were cotransfected with the 2.4 kb fragment and pSVtkneoß. Three of them yielded neo^r transfectants that stimulated TNF release by anti-E CTL clone 82/30, which is CD8+ (Figure 11). No E- transfectant was obtained with four other melanomas, some of which shared A29, B44 or C6 with MZ2. This suggests that the presenting molecule for antigen MZ2-E is HLA-A1. In confirmation, it was found that, out of 6 melanoma cell lines derived from tumors of HLA-A1 patients, two stimulated TNF release by anti-E CTL clone 82/30 of patient MZ2. One of these tumor cell lines, MI13443-MEL, also showed high sensitivity to lysis by these anti-E CTL. These two melanomas were those that expressed MAGE-1 gene (Figure 11). Eight melanomas of patients with HLA haplotypes that did not include A1 were examined for their sensitivity to lysis and for their ability to stimulate TNF release by the CTL. None was found to be positive. The ability of some human anti-tumor CTL to lyse allogeneic tumors sharing an appropriate HLA specificity with the

original tumor has been reported previously (Darrow, et al., J. Immunol. 142: 3329 (1989)). It is quite possible that antigenic peptides encoded by genes MAGE 2 and 3 can also be presented to autologous CTL by HLA-A1 or other class I molecules, especially in view of the similar results found with murine tumors, as elaborated upon supra.

Example 27

As indicated supra, melanoma MZ2 expressed antigens F, D and A', in addition to antigen E. Following the isolation of the nucleic acid sequence coding for antigen E, similar experiments were carried out to isolate the nucleic acid sequence coding for antigen F.

To do this, cultures of cell line MZ2-MEL2.2, an E^- cell line described supra, were treated with anti-F CTL clone 76/6, in the same manner described for treatment with anti-E CTL clones. This resulted in the isolation of an F antigen loss variant, which was then subjected to several rounds of selection. The resulting cell line, "MZ2-MEL2.2.5" was completely resistant to lysis by anti-F CTLs, yet proved to be lysed by anti-D CTLs.

Again, following the protocols set forth for isolation of antigen -E precursor DNA, the F^- variant was transfected with genomic DNA from F^+ cell line MZ2-MEL3.0. The experiments yielded 90,000 drug resistant transfectants. These were tested for MZ2-F expression by using pools of 30 cells in the TNF detection assay elaborated upon supra. One pool stimulated TNF release by anti-F CTLs, and was cloned. Five of 145 clones were found to stimulate anti-F CTLs. Lysis assays, also following protocols described supra, confirmed (i) expression of the gene coding for antigen F, and (ii) presentation of antigen F itself.

Example 28

Following identification of F^+ cell lines, the DNA therefrom was used to transfect cells. To do this, a cosmid library of F^+ cell line MZ2-MEL.43 was prepared,

again using the protocols described supra. The library was divided into 14 groups of about 50,000 cosmids, and DNA from each group was transfected into MZ2-MEL2.2.5. Transfectants were then tested for their ability to stimulate TNF release from anti-F CTL clone 76/6. Of 14 groups of cosmids, one produced two independent transfectants expressing antigen F; a yield of two positives out of 17,500 geniticin resistant transfectants.

Example 29

The existence of a gene family was suggested by the pattern observed on the Southern blot (Figure 12). To do this, the 2.4 kb BamHI fragment, which transferred the expression of antigen MZ2-E, was labelled with 32p and used as a probe on a Southern Blot of BamHI digested DNA of E + cloned subclone MZ2-MEL2.2. Hybridization conditions included 50 μl/cm^2 of 3.5xSSC, 1xDenhardt's solution; 25 mM sodium phosphate buffer (pH 7.0), 0.5% SDS, 2mM EDTA, where the 2.4 kb probes had been labelled with [α^{32}p]dCTP (2-3000 Ci/mole), at 3x10^6 cpm/ml. Hybridization was carried out for 18 hours at 65°C. After this, the membranes were washed at 65°C four times for one hour each in 2xSSC, 0.1% SDS, and finally for 30 minutes in 0.1xSSC, 0.1% SDS. To identify hybridization, membranes were autoradiographed using Kodak X-AR film and Kodak X-Omatic fine intensifying screens.

In the following examples, whenever "hybridization" is referred to, the stringency conditions used were similar to those described supra. "Stringent conditions" as used herein thus refers to the foregoing conditions; subject to routine, art recognized modification.

Example 30

The cDNA coding for MAGE 4 was identified from a sample of the human sarcoma cell line LB23-SAR. This cell line was found to not express MAGE 1, 2 or 3, but the mRNA of the cell line did hybridize to the 2.4 kb sequence for

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MAGE 1. To study this further, a cDNA library was prepared from total LB23-SAR mRNA, and was then hybridized to the 2.4 kb fragment. A cDNA sequence was identified as hybridizing to this probe, and is identified hereafter as MAGE 4.

Example 31

Experiments were carried out using PHA-activated lymphocytes from patient "MZ2", the source of the "MZ" cells discussed supra. An oligonucleotide probe which showed homology to MAGE 1 but not MAGE 2 or 3 was hybridized with a cosmid library derived from the PHA activated cells. The size of the hybridizing BamHI cosmid fragment, however, was 4.5 kb, thus indicating that the material was not mage 1; however, on the basis of homology to mage 1-4, the fragment can be referred to as "MAGE 5". The sequence of MAGE 5 is presented in SEQ ID NO: 16.

Example 32

Melanoma cell line LB-33-MEL was tested. Total mRNA from the cell line was used to prepare cDNA, which was then amplified with oligos CHO9: (ACTCAGCTCCTCCCAGATTT) (SEQ ID NO: 53), and CHO10: (GAAGAGGAGGGGCCAAG) (SEQ ID NO: 54). These oligos correspond to regions of exon 3 that are common to previously described mage 1, 2 and 3.

To do this, 1 μg of RNA was diluted to a total volume of 20 μl, using 2 μl of 10x PCR buffer, 2 μl of each of 10 mM dNTP, 1.2 μl of 25 mM $MgCl_2$, 1 μl of an 80 mM solution of CHO9, described supra, 20 units of RNAsin, and 200 units of M-MLV reverse transcriptase. This was followed by incubation for 40 minutes at 42°C. PCR amplification followed, using 8 μl of 10x PCR buffer, 4.8 μl of 25 mM $MgCl_2$, 1 μl of CHO10, 2.5 units of Thermus acquaticus ("Taq") polymerase, and water to a total volume of 100 μl. Amplification was then carried out for 30 cycles (1 minute 94°C; 2 minutes at 52°C, 3 minutes at 72°C). Ten μl of each reaction were then size fractionated on agarose gel,

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followed by nitrocellulose blotting. The product was found to hybridize with oligonucleotide probe CHO18 (TCTTGTATCCTGGAGTCC) (SEQ ID NO: 55). This probe identified mage 1 but not mage 2 or 3. However, the product did not hybridize to probe SEQ 4 (TTGCCAAGATCTCAGGAA) (SEQ ID NO: 56). This probe also binds mage 1 but not 2 and 3. This indicated that the PCR product contained a sequence that differed from MAGE 1, 2 and 3. Sequencing of this fragment also indicated differences with respect to MAGE 4 and 5. These results indicate a sequence differing from previously identified MAGE 1, 2, 3, 4 and 5, and is named MAGE 6.

Example 33

In additional experiments using cosmid libraries from PHA-activated lymphocytes of MZ2, the 2.4 kb MAGE 1 fragment was used as a probe and isolated a complementary fragment. This clone, however, did not bind to oligonucleotides specific for MAGE 1, 2, 3 or 4. The sequence obtained shows some homology to exon 3 of mage 1, and differs from MAGES 1-6. It is referred to as mage 7 hereafter. Additional screenings yielded MAGE 8-11. All MAGE sequences identified are presented as SEQ ID's.

Example 34

The usefulness of the TRAPs, as well as TRAs derived therefrom, was exemplified by the following.

Exon 3 of MAGE 1 was shown to transfer expression of antigen E. As a result, it was decided to test whether synthetic peptides derived from this exon 3 could be used to confer sensitivity to anti-E CTL.

To do this, and using standard protocols, cells normally insensitive to anti-E/CTLs were incubated with the synthetic peptides derived from Exon 3.1. Using the CTL lytic assays described supra on P815A, and a peptide concentration of 3 mM, the peptide Glu-Ala-Asp-Pro-Thr-Gly-His-Ser-Tyr (SEQ ID NO: 26) was shown to be best. The

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assay showed lysis of 30%, indicating conferring of sensitivity to the anti-E CTL.

Example 35

Nucleic acid sequences referred to as "smage" were isolated from murine cells. Using the protocols described supra, a cosmid library was prepared from the DNA of normal DBA/2 kidney cells, using cosmid vector C2RB. As a probe, the 2.4 kb BamHI fragment of MAGE-1 was used. The DNA was blotted to nylon filters, and these were washed in 2xSSC at 65°C to identify the smage material.

Example 36

Further tissue samples were tested for the presence of MAGE genes, using the protocols discussed supra. Some of these results follow.

There was no expression of the MAGE genes in brain or kidney tumor tissue. Colon tumor tissue showed expression of MAGE 1, 2, 3 and 4, although not all tumors tested showed expression of all MAGE genes. This is also true for pancreatic tumor (MAGE 1); non-small cell lung (MAGE 1, 2, 3 and 4), prostate (MAGE 1), sarcomas (MAGE 1, 2, 3 and 4), breast (MAGE 1, 2 and 3), and larynx (MAGE 1 and 4).

Example 37

A cytolytic CTL clone "20/38" was obtained from peripheral blood lymphocytes of melanoma patient MZ2. This clone is described by Van den Eynde et al., Int. J. Cancer 44: 634-640 (1989), the disclosure of which is incorporated by reference. The CTL clone was isolated following Herin et al., Int. J. Cancer 39: 390-396 (1987), which is incorporated by reference. The assay is described herein, however. Autologous melanoma cells were grown in vitro, and then resuspended at 10^7 cells/ml in DMEM, supplemented with 10% HEPES and 30 mM FCS, and incubated for 45 minutes at 37°C with 200 μCi/ml of $Na(^{51}Cr)O_4$. Labelled cells were washed three times with DMEM, supplemented with 10 mM

HEPES. These were then resuspended in DMEM supplemented with 10 mM HEPES and 10% FCS, after which 100 μl aliquots containing 10^3 cells, were distributed into 96 well microplates. Samples of the CTL clone were added in 100 ul of the same medium, and assays were carried out in duplicate. Plates were centrifuged for four minutes at 100g, and incubated for four hours at 37°C in a 5.5% CO_2 atmosphere.

Plates were centrifuged again, and 100 ul aliquots of supernatant were collected and counted. Percentage of ^{51}Cr release was calculated as follows:

$$\% \ ^{51}Cr \text{ release} = \frac{(ER-SR)}{(MR-SR)} \times 100$$

where ER is observed, experimental ^{51}Cr release, SR is spontaneous release measured by incubating 10^3 labeled cells in 200 ul of medium alone, and MR is maximum release, obtained by adding 100 ul 0.3% Triton X-100 to target cells.

Those mononuclear blood samples which showed high CTL activity were expanded and cloned via limiting dilution, and were screened again, using the same methodology.

The same method was used to test target K562 cells. When EBV-B cells were used, the only change was the replacement of DMEM medium by Hank's medium, supplemented with 5% FCS.

These experiments led to isolation of CTL clone 20/38.

Figure 14 presents the results of these assays. Specifically, it will be seen that the CTL clone lysed autologous melanoma cell line MZ2-MEL.3.0, but did not lyse EBV-B cell lines, fibroblasts, K562 or non-autologous melanoma cell line SK-MEL-29.

Exampl 38

Once the CTL clone was recognized as being specific for the autologous cell line, it was tested for antigenic

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specificity. To do this, antigen loss variants derived from melanoma cell line MEL-MZ2 were tested in the same type of chromium release assay described above. These target lines were MZ2-MEL 3.0, which is D^+, E^+, F^+, A^+, MZ2-MEL.61, which is D^-, MZ2-MEL 2.2, which is E^-, and MZ2-MEL.4, which is F^-. In addition to CTL clone 20/38, clones which are known to be anti-A (CTL 28/336), anti-F (CTL 76/6), and anti-E (CTL 22/13) were tested.

These results are set forth in figure 15. It will be seen that CTL clone 20/38 lysed all the cell lines leading to chromium release except D^- cell line MZ2-MEL.61, thus indicating that the CTL clone is anti-D. This result was confirmed, in experiments not included herein, by experiments where TNF release by the CTL clone was observed only in the presence of melanoma lines presenting antigen D.

Example 39

Once antigen D was identified as the target molecule, studies were carried out to determine the HLA type which presented it. The experiments described in example 38 showed that antigen D was presented by MZ2-MEL, and this cell line's HLA specificity is known (i.e., A1, A29, B37, B44, Cw6, C.cl.10). It was also known, however, that a variant of MZ2-MEL which had lost HLA molecules A29, B44 and C.cl.10 still expressed antigen D, so these could be eliminated from consideration. Studies were not carried out on lines expressing B37, as none could be found.

In all, 13 allogeneic lines were tested, which expressed either HLA-A1 (10 of 13), or Cw6 (3 of 13). The cell lines were tested for their ability to stimulate release of TNF by CTL clone 20/38, using the method of Traversari et al., Immunogenetics 35: 145-152 (1992), the disclosure of which is incorporated by reference. This assay measures TNF release via testing toxicity of supernatants on WEHI 164-13 cells.

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In the assays, cell samples (3000, 10,000 or 30,000 cells) from the allogeneic lines were cultured in the presence of 1500 cells of the CTL clone, and 25 u/ml of IL-2. Twenty-four hours later, the supernatant from the culture was tested against the WEHI cells for toxicity. The results are presented in Table 3, which follows.

Eight cell lines were found to stimulate TNF release from the CTL clone 20/38. All of these lines were HLA-A1. None of the Cw6 presenting lines did so.

The cell lines were also assayed to determine MAGE expression. All eight of the lines which stimulated TNF release expressed MAGE-3, whereas the two HLA-A1 lines which were negative did not.

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Table 3

Melanoma	Number of Cells	TNF pg/ml Exp 1		TNF pg/ml Exp 2		Expression of Mage-3	Expression of HLA-A-1
			+CTL 20/38		+CTL 20/38		
MZ2-MEL.61.2	50000		1		4	+++	+
MZ2-MEL-ET1	50000		>120		>120	+++	+
	1666		66		>120		
LY-1-MEL	30000	1	>120	1	>120	+++	+
	10000	1	>120	1	>120		
	3000	<1	114	2	>120		
MI-10221	30000	<1	>120			+++	+
	10000	<1	71				
	3000	<1	74				
LY-2-MEL	30000	1	57			+++	+
	10000	1	86				
	3000	1	91				
LY-4-MEL	30000	1	>120			+++	+
	10000	1	>120				
	3000	1	>120				
SK23-MEL	30000	1	112			+++	+
	10000	1	116				
	3000	1	105				
MI-665/2-MEL	30000	1	3	2	4	-	+
	10000	1	2	2	5		
	3000	1	5,2	1	5		
LB34-MEL	30000	1	>120			+++	+
	10000	1	>120				
	3000	1	>120				
LB45-MEL	30000	1	11	1	30	-	+
	10000	1	6	1	12		
	3000	1	2	<1	7		
NA-6-MEL	30000	1	77	5	98	+++	+
	10000	1	104	5	>120		
	3000	1	110	4	>120		
MI-13443-MEL	30000	1	>120			+++	+
	10000	1	>120				
	3000	1	>120				
LB5-MEL	30000	1	8	4	9	+	-
	10000	<1	5	4	11		
	3000	<1	5	1	5		
SK64-MEL	30000	1	4	2	5	?	-
	10000	1	2	1	5		
	3000	1	1	1	4		
LB33-MEL	30000			1	3,5	+++	-
	10000			1	4		
	3000			1	3		
LB73-MEL	50000		16			-	-

1500 CTL 20/38 and 25µ/ml IL2 were mixed with the indicated number of cells of the different allogeneic melanomas. 24 hours later, the amount of TNF present in the supernatant was assayed by testing its cytotoxicity for WEHI-164-13 cells.

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Example 40

In view of the results set forth in example 39, experiments were carried out to determine if antigen D was in fact a tumor rejection antigen derived from MAGE-3. To do this, recipient COS-7 cells were transfected with 100ng of the gene for HLA-A1 cloned into pcDNA I/Amp, and 100 ng of one of (a) cDNA for MAGE-1 cloned into pcDNA I/Amp, (b) cDNA for MAGE-2 cloned into pcDSRα, or (c) cDNA for MAGE-3 cloned into pcDSRα. The transfecting sequences were ligated into the plasmids in accordance with manufacturer's instructions. Samples of COS-7 cells were seeded, at 15,000 cells/well into tissue culture flat bottom microwells, in Dulbeco's modified Eagles Medium ("DMEM") supplemented with 10% fetal calf serum. The cells were incubated overnight at 37°C, medium was removed and then replaced by 30 μl/well of DMEM medium containing 10% Nu serum, 400 μg/ml DEAE-dextran, 100 μM chloroquine, and the plasmids described above. Following four hours of incubation at 37°C, the medium was removed, and replaced by 50 μl of PBS containing 10% DMSO. This medium was removed after two minutes and replaced by 200 μl of DMEM supplemented with 10% of FCS.

Following this change in medium, COS cells were incubated for 24 hours at 37°C. Medium was then discarded, and 1500 cells of CTL clone 20/38 were added, in 100 μl of Iscove's medium containing 10% pooled human serum, supplemented with 25 u/ml of IL-2. Supernatant was removed after 24 hours, and TNF content was determined in an assay on WEHI cells, as described by Traversari et al., Immunogenetics 35: 145-152 (1992), the disclosure of which is incorporated by reference. These results are shown in figure 16.

It will be seen that the CTL clone was strongly stimulated by COS-7 cells transfected with HLA-A1 and MAGE-3, but not by the cells transfected with the other Mage genes. This leads to the conclusion that antigen D is a tumor rejection antigen derived from the tumor rejection

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antigen precursor coded by gene MAGE-3, and that this TRA is presented by HLA-A1 molecules.

Example 41

It is well known that different alleles of genes may produce different proteins. This principle should extend to the MAGE family of genes as well, and is an important consideration in view of diagnostic and therapeutic ramifications. Thus, polymorphism in the MAGE family was studied.

To address the issue of polymorphism, blood lymphocytes of ten individuals were collected, and genomic DNA extracted. This DNA was subjected to Southern blotting in accordance with James et al., Canc. Res. 48: 5546-5551 (1988), incorporated by reference. Briefly, the labelled 2.4 kb genomic DNA fragment of MAGE-1, containing the last two exons of MAGE-1, described supra, was hybridized with the filter carrying the digested DNA, at 42°C for at least 16 hours, in 50% formamide, 5% dextran sulfate, 6xSSC, 1% SDS and 0.1 mg/ml heterologous DNA. The hybridization filters were washed, consecutively, in 2xSSC, 0.1% SDS (room temperature, 15 minutes), and twice in 0.1xSSC, 0.1% SDS at 67°C for 30 minutes, each wash. Autoradiography was carried out at -70°C for 7-10 days, using standard film.

A pattern of 13 hybridizing bands was observed, which was conserved over all individuals. One individual did show an additional band, but also showed the 13 band pattern.

Example 42

It was of interest to determine which chromosome or chromosomes bear the MAGE genes. To ascertain this, a panel of hamster/human somatic cell hybrids was used. The hybrids were obtained either from the Human Genetic Mutant Cell Repository ("GM" prefix), or from Johns Hopkins University ("A_3" prefix). Each hybrid was cytogenetically studied to determine human chromosome content.

Total genomic DNA of the hybrids was probed in the same manner described in Example 41, supra (the conditions of stringency used prevented cross hybridization with hamster DNA).

Table 4, which follows, summarizes the result of the probe work. Analysis of the data led to the conclusion that the pattern of hybridization was only concordant with location of MAGE-1 on the X chromosome.

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TABLE 4 - Segregation of MAGE-1 with human chromosomes in human-hamster hybrid cell DNA

Hybrid	*MAGE-1*	Human chromosome																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
GM06317	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
GM06318B	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
GM07300	+	-	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-
GM07301	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-
GM08854	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
GM09142	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	2	-
GM10095	+	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-
GM10115	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GM10156B	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GM10253	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GM10322	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-
GM10478	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
GM10479	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
GM10498	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
GM10567	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
GM10611	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GM10612	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
GM10629	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GM10791	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GM10880	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
GM10888	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
A_3ADA ID12	-	+	-	+	+	+	-	-	+	-	-	-	+	+	+	-	+	-	-	-	+	+	-	-	+
A_3ADA6F5	+	+	+	-	-	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	-	+	+	+	+
A_3ADA13	+	+	-	+	+	-	-	+	+	-	-	+	+	+	+	+	±	-	-	+	-	+	-	+	+
A_3ADA14	-	-	-	+	+	+	-	-	+	-	+	+	+	+	+	±	-	-	-	-	+	+	+	-	+
A_3G1	+	-	-	+	+	-	6	-	±	+	+	-	-	-	-	±	-	+	-	±	+	-	±	+	+
A_3HR20	-	-	-	+	+	+	+	-	-	+	-	+	-	-	+	-	-	+	-	-	-	-	±	-	-
P_gMe4	+	-	5	+	-	-	+	+	+	-	-	+	+	+	+	+	-	+	-	-	±	+	-	±	+
Number of concordant hybrids	(+/+)	2	1	3	2	1	3	3	4	2	1	3	4	3	3	3	0	3	1	2	1	3	1	8	4
	(-/-)	17	19	14	14	15	18	18	16	17	18	17	17	16	14	18	17	17	19	18	16	14	15	18	17
Number of discordant hybrids	(+/-)	6	6	5	6	7	3	5	3	6	7	5	4	5	5	4	7	5	7	5	6	5	6	0	4
	(-/+)	2	0	5	5	4	2	1	3	2	1	2	2	3	5	0	2	2	0	1	3	5	3	0	2
Percent discordancy		22	23	38	37	41	24	22	23	30	30	26	22	30	37	16	33	26	27	23	35	37	36	0	26

+ = chromosome present; - = chromosome absent;
± = very faint bands, indicating that only a small percentage of the cells contained the chromosome (not included in calculation of percent discordancy
1,2 - GM09142 contains only part of chromosomes X and 21, der 21 1(X;21) (p21;p12)
3,4 - GM10095 contains only part of chromosomes X and 9, der 9 1(X;9) (q13;q34)
5 - PgMe4 contains a deleted chromosome 2 and is missing 2p23-p24
6 - A_3G1 contains only the q arm of chromosome 6

Example 43

In this experiment, a study was carried out to determine if all twelve known MAGE genes were located on the X chromosome. This was accomplished via the use of polymerase chain reaction ("PCR") technology.

RNA purification and cDNA synthesis were first carried out, in accordance with Weynants et al., Int. J. Cancer 56: 826-829 (1994), incorporated by reference herein. Next, 1/20 of the cDNA produced from 2 ug of total RNA was supplemented with 5 ul of PCR buffer (500 mM KCl, 100 mM Tris pH 8.3), 1 ul each of 10 mM dNTPs, 25 pmoles of each primer (see below), 3 ul of 25 mM $MgCl_2$, and 1.25 units of Taq polymerase, with water added to final volume of 50 ul.

The primers were as follows:

MAGE-3: 5'-TGGAGGACCAGAGGCCCCC, 5'-GGACGATTATCAGGAGGCCTGC (725 bp) (SEQ ID NOS: 27 AND 28)

MAGE-4: 5'-GAGCAGACAGGCCAACCG, 5'-AAGGACTCTGCGTCAGGC (446 bp) (SEQ ID NOS; 29 AND 30)

MAGE-5: 5'CTAGAGGAGCACCAAAGGAGAAG, 5'-TGCTCGGAACACAGACTCTGG (413 bp) (SEQ ID NOS: 31 AND 32)

MAGE-6: 5'-TGGAGGACCAGAGGCCCCC, 5'-CAGGATGATTATCAGGAAGCCTGT (727 bp) (SEQ ID NOS: 33 AND 34)

MAGE-7: 5'-CAGAGGAGCACCGAAGGAGAA, 5'-CAGGTGAGCGGGGTGTGTC (405 bp) (SEQ ID NOS: 35 AND 36)

MAGE-8: 5'-CCCCAGAGAAGCACTGAAGAAG, 5'-GGTGAGCTGGGTCCGGG (399 bp) (SEQ ID NOS: 37 AND 38)

MAGE-9: 5'-CCCCAGAGCAGCACTGACG, 5'-CAGCTGAGCTGGGTCGACC (391 bp) (SEQ ID NOS: 39 AND 40)

MAGE-10: 5'-CACAGAGCAGCACTGAAGGAG, 5'-CTGGGTAAAGACTCACTGTCTGG (485 bp) (SEQ ID NOS: 41 AND 42)

MAGE-11: 5'-GAGAACCCAGAGGATCACTGGA, 5'-GGGAAAAGGACTCAGGGTCTATC (422 bp) (SEQ ID NOS: 43 AND 44)

MAGE-12: 5'-GGTGGAAGTGGTCCGCATCG, 5'-GCCCTCCACTGATCTTTAGCAA (392 bp) (SEQ ID NOS: 45 AND 46)

Amplification was carried out for 30 cycles (MAGE-3, 4, 6, 12) or 32 cycles (MAGE-5, 7-11), where a cycle was one minute at 94°C followed by two minutes at 65°C for MAGE-5, 7-12, or two

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minutes at 68°C (MAGE-4), or two minutes at 71°C (MAGE-3 and MAGE-6); followed by three minutes at 72°C (MAGE-3, 5-12), or two minutes at 72°C (MAGE-4). The analysis was carried out on hybrid cell line GM 10868, which contains human chromosome 12, and GM 07301, which contains chromosome 12 and the X-chromosome. All assays were negative with the human GM 10868 line, and all were positive with the GM 07301 cell line, which indicated that all 12 genes are found on the X-chromosome.

Example 44

The sizes of mRNAs for the different MAGE genes are similar, and thus Northern blot analysis cannot be used to determine expression of the various MAGE genes in different tissues, both normal and tumor. PCR analysis, along the lines of the study in example 43, supra, however, was believed to be useful.

To this end, a series of various tumors and normal tissues were tested for expression of MAGE genes.

Total RNA of the cells tested was extracted, and was then oligo dT primed, following art known techniques. The resulting material was then subjected to PCR, following the protocols of example 43, supra. For MAGE-1 and MAGE-2, the protocols of Brasseur et al., Int. J. Cancer. 52: 839-841 (1992), and DeSmet et al., Immunogenetics 39: 121-120 (1994), both of which are incorporated by reference, were used.

Table 5, which follows, elaborates these results, with a representative but by no means exhaustive listing of tissues tested. Each of MAGE 1-4, 6 and 12 showed significant expression in a number of tumors of varied tissue types. MAGE-5 and 8-11 were expressed very weakly in all tissues tested, whereas MAGE-7 RNA was not detectable at all. With respect to normal tissues, including tissues taken from a >20 week fetus, all were negative for MAGE RNA but for testis and placenta. Testis expressed all MAGE genes but MAGE-7, while placenta expressed MAGE-3, 4, and 8-11.

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TABLE 5. Expression of Mage-1, 2, 3, 4, 6 and -12 by tumors and normal tissues

	MAGE 1	MAGE 2	MAGE 3	MAGE 4	MAGE 6	MAGE 12
COLON CARCINOMAS						
MZ-CO-2 ¶	++	++	+	-	-	+
SK-CO-11 ¶	-	++	+++	-	+	++
LB150 **	-	-	-	+	-	-
HSR 320 ¶	-	+++	+++	+	++	+++
LEUKEMIAS						
K562 ¶	-	++	+++	-	++	+++
MELANOMAS						
MI10221 ¶	-	+++	+++	+++	+++	+++
MZ2-MEL 3.0 ¶	+++	+++	+++	-	+++	+
LB265 **	-	++	-	-	-	+
LG7 **	-	++	-	-	-	-
LG11 **	++	++	++	-	-	+++
LB271 **	-	++	+++	-	++	+++
LUNG CANCERS						
LB178 (NSCLC) **	++	-	-	+++	-	-
LB175 (NSCLC) **	-	++	+++	+++	-	+++
LB11 (SCLC) ¶	++	+++	+++	-	-	+++
LB12 (SCLC) ¶	-	+++	+++	-	-	+++
SARCOMAS						
LB23 ¶	-	-	-	++	-	-
LB408 **	-	-	-	++	-	-
LB258 **	+	++	+	-	-	++
BREAST CARCINOMAS						
LB280 **	++	-	++	-	-	+
LB284 **	++	++	++	+	-	++
Stomach	-	-	-	-	-	-
Lung	-	-	-	-	-	-
Breast	-	-	-	-	-	-
Colon	-	-	-	-	-	-
Skin	-	-	-	-	-	-
Uterus	-	-	-	-	-	-
Testis	++	++	++	++	++	++
Thymocytes	-	-	-	-	-	-
EBV-lymphocytes	-	-	-	-	-	-
Foetal liver	-	-	-	-	-	-
Foetal brain	-	-	-	-	-	-
Placenta LB694	-	-	+	+++	-	-

RNA from tumor cell lines (¶), tumor samples (**) and normal tissues were tested by RT-PCR for the expression of MAGE genes. PCR primers were chosen as indicated in methods. For MAGE-12, PCR amplification of RNA in the absence of reverse transcription indicated that in our conditions the contamination by genomic DNA was negligible. The level of expression evaluated by band intensity of PCR products fractionated in agarose gels is represented by +++, ++, +. Absence of product is indicated by -.

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Exampl 45

The expression of the MAGE-1, 2 and 3 genes in various tumors and normal tissues was evaluated, using both reverse transcription and polymerase chain reaction ("PCR") amplification. To perform these assays, the total RNA of the cells of interest was extracted via the well known guanidine-isothiocyanate procedure of Davis et al., Basic Methods in Molecular Biology, 1986 (New York, Elsevier, pp. 130), which is incorporated by reference in its entirety. cDNA was then synthesized, by taking 2 ug of the RNA, diluting it with water, and then adding the following materials: 4 ul of 5X reverse transcriptase buffer, 1 ul each of each dNTP (10 mM), 1 ul of a 40 μM solution of oligo dT(15), 20 units of RNAsin, 2 ul of 0.1 M dithiothreitol, and 200 units of MoMLV reverse transcriptase. All materials were mixed in a 20 ul reaction volume, and incubated at 42°C for 60 minutes and diluted to 100 ul with water.

Presence or absence of each of MAGE-1, -2, and -3 cDNA was detected via PCR amplification, in separate reactions, using oligonucleotide primers located in different exons of the MAGE gene of interest. For MAGE-1, the primers were:

5'-CGGCCGAAGGAACCTGACCCAG-3'
(SEQ ID NO: 47)

5'-GCTGGAACCCTCACTGGGTTGCC-3'
(SEQ ID NO: 48)

These are described by Brasseur et al., Int. J. Cancer 52: 839-841 (1992).

For MAGE-2, the primers were:

5'-AAGTAGGACCCGAGGCACTG-3'
(SEQ ID NO: 49)

5'-GAAGAGGAAGAAGCGGTCTG-3'
(SEQ ID NO: 50)

(DeSmet et al., Immunogenetics 39: 121-129 (1994)).

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For MAGE-3, the primers were:

5'-TGGAGGACCAGAGGCCCCC-3'
(SEQ ID NO: 27)

5'-GGACGATTATCAGGAGGCCTGC-3'
(SEQ ID NO: 28)

(Serial No. 08/204,727 filed March 1, 1994 to Gaugler et al. incorporated by reference).

For each PCR reaction, 5 ul of cDNA were supplemented with 5 ul of 10XPCR buffer, 1 ul of each dNTP (10 mM), 1 ul each of 40 μM primer solutions, 1.25 units of Taq polymerase, and water, to a total volume of 50 ul. Each mixture was heated for five minutes at 94°C. Amplification was then carried out for 30 cycles (MAGE-1: 1 minute at 94°C, 3 minutes at 72°C; MAGE-2: 1 minute at 94°C, 2 minutes at 67°C; MAGE-3: 1 minute at 94°C, 4 minutes at 72°C). Cycling was concluded, in each case, with a final extension at 72°C for 15 minutes. A 10 ul sample of each reaction was run on a 1% agarose gel, and visualized by ethidium bromide fluorescence. To ensure that RNA was not degraded, a PCR assay with primers specific for ß-actin was carried out, following the listed protocols, except that only 20 cycles were carried out with the annealing step at 65°C. Date are summarized in the Table which follows:

Table 6 **Expression of gene MAGE-1, 2 and 3 in lung tumors**

	Proportion of positive samples		
	MAGE-1	MAGE-2	MAGE-3
Non-small cell lung cancer	16/46	16/46	14/46
squamous cell carcinoma	8/26	6/26	7/26
adenocarcinoma	8/18	9/18	7/18
large cell carcinoma	0/2	1/2	0/2
Small cell cancer	1/3	2/3	2/3
Normal lung samples	0/8	0/8	0/8

Example 46

The previous example showed how to identify expression of various MAGE genes. This example explains quantitation of the expression.

First, cDNA was synthesized in the same way described in example 1, except that the oligo dT consisted solely of dT15, and the reaction mixture was preincubated at room temperature for 10 minutes to optimize annealing. Also, following the incubation, the transcriptase activity was terminated by heating the mixture at 95°C for 15 minutes. PCR amplification was carried out, by combining 5 ul of 10x PCR buffer, 0.5 ul of a 2.5 mM dNTP mix, 0.2 μCi of α^{32}P-dCTP, 0.5 ul of each primer (40 μM solution), 1.25 units of Taq polymerase, and water, to a total of 50 ul. The mixtures were chilled on ice, and then 5 ul of chilled cDNA solution (100 ng total RNA) were added thereto. The

mixture was heated to 94°C for five minutes, and 24 cycles of amplification were carried out (one minute at 94°C, three minutes at 72°C per cycle). Cycling concluded with a final extension at 72°C, for 15 minutes. A 15 ul sample of PCR product was run on an agarose gel which was then fixed in 10% trichloroacetic acid for 30 minutes, dried, and then exposed to a phospho-screen for 90 minutes before scanning by Phosphor-Imager to measure incorporated ^{32}P. This was compared to the incorporations from various dilutions of RNA of reference melanoma cell line MZ2-MEL-3.0.

Quantitative measurements of ß-actin messenger and "GAPDH" (i.e., glyceraldehyde 3-phosphate dehydrogenase) was carried out on each cDNA sample, under similar conditions. The one difference was that only 18 amplification cycles were carried out. A separate PCR reaction was set up with primers for ß-actin and GAPDH, with only ß-actin used for normalization. Results were expressed via formula:

$$100 \times \frac{\left[\frac{\text{MAGE-1-S}}{\text{Actin-S}}\right]}{\left[\frac{\text{MAGE-1-MEL}}{\text{Actin-MEL}}\right]}$$

where: S = product from tumor sample
MEL = product from MZ2-MEL 3.0

The results obtained were comparable to those obtained previously with melanoma tumors. Level of expression varied, from 1 to 160% of the amount expressed by the reference cell line. Figure 2 presents some of these results (i.e., normalized results, relative to levels of ß-actin expression). Values are percent of the level of MAGE-1 expression measured with RNA of the reference line

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MZ2-MEL-3.0. Values are for MAGE-1 positive tumors of Table 2). Table 7, which follows, summarizes patterns of expression for various tumors.

Table 7. Pattern of expression of genes MAGE-1,2 and 3 by MAGE-positive lung tumor samples

	MAGE-1*	MAGE-2	MAGE-3
Squamous cell carcinoma			
LB 175	++	++	+++
LB 178	++	-	-
LB 182 (A1)*	-	+	-
LB 195	+	++	+++
LB 206	+++	+	++
LB 321	+	-	-
LB 323	+++	+	+++
LB 424	+	-	+
LB 425	-	-	+
LB 498 (A1)	+++	-	-
LB 557	-	+++	+++
Adenocarcinoma			
LB 117 (A1)	+	++	++
LB 212	++	+	-
LB 264 (A1)	+++	++	+++
LB 292	-	++	+++
LB 306	++	+	++
LB 322	-	+	+
LB 474 (A1)	+	++	-
LB 497	++	+++	+++
LB 510	+++	-	-
LB 558 (A1)	+	+	+
Large cell carcinoma			
LB 259	-	+	-
Small cell lung cancer			
LB 444	-	++	+++
LB 648 (A1)	+	++	+++

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Exampl 47

The expression of the MAGE-3 gene in various tumors and normal tissues was evaluated, using both reverse transcription and polymerase chain reaction ("PCR") amplification. To perform these assays, the total RNA of the cells of interest was extracted via the well known guanidine-isothiocyanate procedure of Davis et al., Basic Methods in Molecular Biology, 1986 (New York, Elsevier, pp. 130), which is incorporated by reference in its entirety. cDNA was then synthesized, by taking 2 ug of the RNA, diluting it with water, and then adding the following materials: 4 ul of 5X reverse transcriptase buffer, 1 ul each of each dNTP (10 mM), 2 ul of a 20 μM solution of oligo dT, 20 units of RNAsin, 2 ul of 0.1 M dithiothreitol, and 200 units of MoMLV reverse transcriptase. All materials were mixed in a 20 ul reaction volume, and incubated at 42°C for 60 minutes. For the amplification reaction, 1/20 of the cDNA reaction product was supplemented with 5 ul of PCR buffer, 0.5 ul of each of the dNTPs (10 mM), 1 ul each of 20 μM solutions of primer (see infra), and 1.25 units of Taq polymerase. Water was added to a final volume of 50 uls. The primers used for MAGE-3 were:

5'-TGGAGGACCAGAGGCCCCC-3'
(SEQ ID NO: 27)

5'-GGACGATTATCAGGAGGCCTGC-3'
(SEQ ID NO: 28)

These correspond to a sense sequence in exon 2 of the gene (SEQ ID NO: 27), and an antisense sequence in exon 3 (SEQ ID NO: 28).

PCR was performed for 30 cycles (one minute at 94°C, four minutes at 72°C). PCR products were size fractionated on a 1% agarose gel, and then analyzed. The results are presented in the table which follows. These data confirm

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some results obtained previously, but also show the expression of MAGE-3 in head and neck squamous cell carcinomas, a result not suggested by previous work.

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Table 8. Expression of gene MAGE-3 by tumoral, normal and fetal tissues.

TUMORS

HISTOLOGICAL TYPE	Number of MAGE-3 positive tumors*	
	cell lines	*tumors samples*
Melanomas	50/62 (81%)	72/105 (69%)
Head and neck squamous cell carcinomas	-	20/36 (56%)
Lung carcinomas		
NSCLC ‡	1/2	14/46 (30%)
SCLC	18/22 (82%)	2/3
Colorectal carcinomas	5/16	5/31 (16%)
Mammary carcinomas	2/6	16/132 (12%)
Bladder tumors	-	2/6
Sarcomas	1/4	3/10
Prostatic carcinomas	-	3/20
Renal carcinomas	0/5	0/38
Leukemias	2/6	0/20
Lymphomas	0/6	0/5

NORMAL TISSUES

HISTOLOGICAL TYPE	MAGE-3 expression*
ADULT TISSUES	
Brain	-
Colon	-
Stomach	-
Liver	-
Ovary	-
Skin	-
Lung	-
Kidney	-
Breast	-
Testis	++
FETAL TISSUES	
Brain	-
Liver	-
Spleen	-

*Expression of gene MAGE-3 was tested by RT-PCR amplification on total RNA, with the primers described in methods. These primers distinguish MAGE-3 from the 11 other MAGE genes that have been identified.

‡ NSCLC are non-small cell lung carcinomas, SCLC are small cell lung carcinomas.

Example 48

Bladder tumor specimens were collected at surgery. They were divided into two portions, one of which was used for routine histopathological evaluation. The other portion was frozen in liquid nitrogen immediately after transurethral resection, or radical cystectomy. These frozen samples were stored at -80°C until used for RNA extraction. Normal bladder tissue was obtained by biopsies of cadavers from donors in an organ transplant program.

Total RNA was extracted from the samples by the classic guanidine-isothiocyanate/cesium chloride method of Davis et al, Basic Methods in Molecular Biology, pp. 130-135, Elsevier, New York (1986). Synthesis of cDNA was then carried out by extension with oligo(dT) using 2 ug of RNA in a 20 ul reaction volume following DeSmet et al., Immunogenetics 39: 121-129 (1994), incorporated by reference herein. Following incubation at 42°C for one hour, the cDNA reaction mixture was diluted to 100 ul with water. Separate polymerase chain reaction amplification were then carried out to determine whether any of MAGE-1, 2, 3 or 4 cDNA were present. The amplifications were carried out using oligonucleotide primers located in different exons of the MAGE genes. PCR amplification was also carried out using primers for HLA-A1.

The primers used were the following:

5'-TGGAGGACCAGAGGCCCCC-3 (sense, exon 2) (SEQ ID NO: 27) and

5'-GGACGATTATCAGGAGGCCTGC-3' (antisense, exon 3) (SEQ ID NO: 28) for MAGE-3

5'-CGGCCGAAGGAACCTGACCCAG-3' (sense, exon 1) (SEQ ID NO: 47) and

5'GCTGGAACCCTCACTGGGTTGCC-3' (anti-sense, exon 3) (SEQ ID NO: 48) for MAGE-1

5'-AAGTAGGACCCGAGGCACTG-3' (sense, exon 2) (SEQ ID NO: 49) and

5'-GAAGAGGAAGAAGCGGTCTG-3' (anti-sense, exon 3) (SEQ ID NO: 50) for MAGE-2

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5′-GAGCAGACAGGCCAACCG-3′ (sense, exon 2) (SEQ ID NO: 29) and

5′-AAGGACTCTGCGTCAGGC-3′ (anti-sense, exon 3) (SEQ ID NO: 30) for MAGE-4

5′-GGGACCAGGAGACACGGAATA-3′ (sense, exon 2) (SEQ ID NO: 51) and

5′-AGCCCGTCCACGCACCG-3′ (anti-sense, exon 3) (SEQ ID NO: 52) for HLA-A1

SEQ ID NOS: 27 and 28 are described by Weynants et al., Int. J. Cancer 56: 826-829 (1994). SEQ ID NOS: 47 and 48 are described in Brasseur et al., Int. J. Cancer 52: 839-841 (1992). SEQ ID NOS: 49 and 50 are disclosed in DeSmet et al., Immunogenetics 39: 121-129 (1994). SEQ ID NOS: 29 and 30 are disclosed in copending application Serial No. 08/299,849 filed September 1, 1994 to DePlaen et al., and incorporated by reference. SEQ ID NOS: 51 and 52 are found in Gaugler et al., J. Exp. Med. 179: 921-930 (1994), as well as the above-identified parent application. All of these references are incorporated by reference.

The amplification protocol was as follows. Each PCR reaction used 5 ul of cDNA, supplemented with 5 ul of 10x PCR buffer, 1 ul each of 10 mM dNTP, 0.5 ul each of 80 uM solutions of primers, 1.25 units of Taq DNA polymerase, and water to achieve a total volume of 50 ul. The mixtures were heated to 94°C for 5 minutes, followed by amplification in a thermal cycler, for 30 cycles. For MAGE-1, 1 cycle was one minute at 94°C followed by three minutes at 72°C. For MAGE-2, one cycle was 94°C for one minute, followed by two minutes at 67°C and two minutes at 72°C. For MAGE-3, one cycle was one minute at 94°C; followed by four minutes at 72°C. For MAGE-4, one cycle was one minute at 94°C, two minutes at 68°C, and two minutes at 72°C. The cycle for HLA-A1 was the same as that for MAGE-4. A 10 ul sample of each reaction was run on a 1% agarose gel, and then visualized by ethidium bromide fluorescence. In order to provide a control for RNA

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integrity, a 20 cycle PCR assay, using primers specific for ß actin, was carried out in each case, following Weynants et al., supra.

The protocols described were developed with certain goals in mind. Primers were selected so as to be in different exons, thus preventing false positives due to DNA contamination of the RNA preparations. Under the conditions used, DNA generates either no PCR product, or longer products which are readily distinguishable from amplified cDNA. This is shown by figure 19. In figure 19, a bladder tumor sample from a patient, referred to as "HM15" is shown in each "R" lane. Lanes marked "D" show products obtained from amplification of the patients' genomic DNA. The PCR products were run on a 2.5% low melting agarose gel, but the assays were identical to the protocol of this example in all other ways. Size markers are on the left hand side. There was no band in the MAGE-1 reaction, because of the large intron between the two primers.

Table 10, which follows, shows the results obtained for a number of tumors (nomenclature is explained below). Of 57 samples of primary transitional cell carcinoma, 21% expressed MAGE-1, 30% expressed MAGE-2, 35% expressed MAGE-3, and 33% expressed MAGE-4. Ta tumors and low grade T1 tumors expressed none of these, or expressed only a single gene, at low levels. Higher stage tumors, in contrast, frequently expressed high levels of several genes. It was also found that the fraction of invasive tumors which expressed MAGE genes was 2-5 times higher than the fraction observed with superficial tumors, as is depicted in figure 2 (this figure is based upon data from Table 10). Tumors expressing at least one of the four MAGE genes accounted for 61% of the 28 invasive tumors studied. Among the 29 superficial tumors, the proportion was only 28%. Results paralleled other results reported previously for melanoma, in that all but one of the tumors expressing MAGE-1 also expressed MAGE-3.

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None of the six biopsies of normal bladder examined expressed any of the MAGE genes discussed herein.

In some instances, several tumor samples were obtained from the same patient. The analysis of these patients is set out in Table 9. Patient HM61 had a primary tumor and an invaded lymph node. They displayed a very similar pattern of expression of MAGE-1, 2, and 3, with MAGE-1 predominating. Normal mucosa adjacent to the tumor was completely negative for MAGE-2 and MAGE-3, with a very low level of MAGE-1 expression, which was probably due to the presence of a few tumor cells. In patient "HM25", the initial tumor, and an early recurrence, both expressed MAGE-1, 2, 3 and 4. A recurrence which occurred two years after the first displayed a very different pattern, expressing only MAGE-2 and MAGE-3. A similar discordance between primary tumor and recurrence was observed with patient "HM20". Patients HM30 and LB526 showed differences in the pattern of MAGE-expression in different samples of the same primary tumor.

In the tables which follow, "Ta" stands for a superficial lesion, limited to bladder mucosa (also known as "stage Ta"). "Stage T1", or "T1" is used for superficial lesions limited to subepithelial connective tissue. "Stages T2-T4", or "T2-T4" refer to tumors which have invaded bladder muscle. The nomenclature "G1", "G2" and "G3" refers to the degree of differentiation, or histopathological grade. "G1" superficial tumor is well differentiated, while a "G3" tumor is poorly differentiated. See Mostofi et al., "Histological Typing of Urinary Bladder Tumors. WHO International Histological Classification of Tumors" (1973).

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TABLE 9 - EXPRESSION OF GENES MAGE-1, 2, 3 AND 4
IN MULTIPLE SAMPLES FROM BLADDER CARCINOMA PATIENTS

Patients	Samples	Tumor stage and grade	MAGE-1	MAGE-2	MAGE-3	MAGE-4
HM 61	Primary tumor	T2 G3	++	+	+	-
	Metastatic iliac lymph node		+++	+	+	-
	Mucosa adjacent to the tumor		+	-	-	-
HM 25	Primary tumor	T2 G2	+	++	+	++
	Tumor recurrence after 1 month	T2 G2	++	++	++	++
	Tumor recurrence after 2 years	T1 G2	-	+++	+++	-
HM 20	Primary tumor	T1 G1	+	-	-	-
	Tumor recurrence after 2 months	T1 G1	-	-	-	-
HM 30	Primary tumor, 1st sample	T2 G2	-	-	++	-
	Primary tumor, 2nd sample	T2 G2	-	-	-	-
LB 526	Primary tumor, radical cystectomy	T3 G2	+	+	++	+
	Primary tumor, 9-day pre-operative biopsy	T3 G2	+	+	+	-

Table 10. EXPRESSION OF GENES MAGE-1, 2, 3 AND 4 IN BLADDER TRANSITIONAL-CELL CARCINOMA SAMPLES

Tumor Stage and Grade ‡		Patients		MAGE-1	MAGE-2	MAGE-3	MAGE-4 ¶
Superficial tumors (n=29)							
Ta (n=7)	G1	HM 7		-	-	+	-
		HM 32	(A1)*	-	-	-	-
		HM 33	(A1)	-	-	-	-
		HM 49		-	-	-	-
	G2	LB 523		-	-	-	-
		LB 817		-	-	-	-
		LB 818		-	-	-	-
T1 (n=22)	G1	HM 2		-	-	-	-
		HM 6	(A1)	-	-	-	-
		HM 17		-	-	-	-
		HM 20		+	-	-	-
		HM 22		-	-	-	-
		HM 34		-	-	-	-
		HM 35		-	-	-	+
	G2	HM 4		-	+	+	+
		HM 5		-	-	-	-
		HM 9		-	-	-	-
		HM 27		-	-	-	+++
		HM 37		-	-	-	-
		HM 38	(A1)	-	-	-	-
		HM 39		-	-	-	+
		HM 40	(A1)	-	-	-	-
		HM 41		-	-	-	-
	G3	HM 14		++	+++	+++	++
		HM 23		-	-	-	-
		HM 26		-	+++	+++	+++
		HM 42	(A1)	-	-	-	-
		HM 53		-	-	-	-
		LB 767	(A1)	-	-	-	-
Invasive tumors (n=28)							
T2 (n=15)	G2	HM 8		-	-	-	-
		HM 13	(A1)	-	-	-	-
		HM 24	(A1)	+	+++	+++	++
		HM 25		+	++	+	++
		HM 30		-	-	++	-
		LB 796		-	-	-	-
	G3	HM 3	(A1)	-	-	-	-
		HM 10		-	+	++	++
		HM 12		-	+	+	-
		HM 15		+++	+++	+++	+++
		HM 61	(A1)	++	+	+	-
		LB 524	(A1)	-	-	+++	+
		LB 824		-	++	+++	+
		LB 825		+	++	+	+
		LB 831		+	++	+++	++
T3 (n=11)	G2	HM 44		-	-	-	-
		HM 45		-	+++	+++	-
		HM 46	(A1)	-	-	-	-
		LB 526		+	+	++	+
	G3	HM 11		++	+	++	+
		HM 18		-	-	-	-
		HM 21		-	-	-	-
		HM 47		+++	+++	+++	+++
		HM 48		-	-	-	-
		HM 50		+++	+++	+++	+
		HM 52	(A1)	-	-	-	-
T4 (n=2)	G3	HM 1		-	-	-	+
		HM 51		-	-	-	-

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The foregoing examples show that expression of MAGE tumor rejection antigen precursors is correlated to various cancers. One aspect of the invention, then, is a method for determining these cancers by assaying a sample for expression of at least one MAGE tumor rejection antigen precursor. As MAGE genes are nearly without exception expressed only by tumor cells, there can be no question but that expression of a MAGE gene or genes is indicative of cancer. The fact that the cancer is a particular type, such as lung adenocarcinoma, is easily ascertainable, as adenocarcinoma cells have distinct morphologies which are identifiable by the skilled artisan. Similarly, the fact that the tumor of interest is a lung adenocarcinoma as compared to a tumor from a different body part is self evident; one does not find lung adenocarcinoma in, e.g., large intestine tissue. Analogous statements can be made for bladder and other cancers.

The assay for the MAGE genes can take many forms. Most preferably, the assay is done via determining gene expression, such as by determining mRNA transcription products. For example, amplification protocols, including but not being limited to polymerase chain reaction (PCR), and ligase chain reaction (LCR), are preferred. The assay can also be carried out using nucleic acid molecule probes, which are labelled or unlabelled, and which specifically hybridize to sequences characteristic of the MAGE gene of interest. Labelling nucleotide probes is well known to the art, labels including radioactive, fluorescent, chromophoric, magnetic, and other identifiable materials. Antibodies, haptens such as biotin, (strept)avidin, digoxin, digoxigenin, and so forth, can all be used. Non-labelled probes can also be used. In such a case, the probes will form a double stranded molecule with their target. Any remaining single stranded material can be enzymatically digested, and when something remains, it is a sign of MAGE expression. For the case of polymerase chain reaction or other methodologies where a primer or

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primers are required, the molecules represented by SEQ ID NO: 47 and SEQ ID NO: 48 are especially preferred for MAGE-1, SEQ ID NO: 49 and 50 for MAGE-2, SEQ ID NOS: 27 and 28 for MAGE-3 and SEQ ID NOS: 29 and 30 for MAGE-4. Similarly, these molecules are preferred as probes.

Quantitation of MAGE expression is shown herein as well. This is an important feature of the invention because in a given tumor sample (as compared to tumor cell lines) there will always be an undetermined proportion of normal cells.

One may also assay for the expression product of the MAGE gene, e.g., the tumor rejection antigen precursor protein, via assays such as immunoassays. See, e.g., U.S. Patent Application Serial No. 08/190,411 filed February 1, 1994, and Chen, et al., Proc. Natl. Acad. Sci. USA 91(3): 1004-1008 (1994), both of which are incorporated by reference, teaching MAGE-1 specific mAbs.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

BNSDOCID: <WO 9523874A1>

(1) GENERAL INFORMATION:

(i) APPLICANTS: De Plaen, Etienne; Boon-Falleur, Thierry; Lethé, Bernard; Szikora, Jean-Pierre; De Smet, Charles; Chomez, Patrick; Weynants, P.; Brasseur, Francis; Marchand, M.; Gaugler, Béatrice; Van den Eynde, Benoit; van der Bruggen, Pierre; Patard, Jean-Jacques

(ii) TITLE OF INVENTION: Method For Determining A Cancerous Condition by Assaying For Expression Of One Or More Mage Tumor Rejection Antigen Precursors

(iii) NUMBER OF SEQUENCES: 56

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Felfe & Lynch
(B) STREET: 805 Third Avenue
(C) CITY: New York City
(D) STATE: New York
(F) ZIP: 10022

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette, 5.25 inch, 360 kb storage
(B) COMPUTER: IBM
(C) OPERATING SYSTEM: PC-DOS
(D) SOFTWARE: Wordperfect

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Hanson, Norman D.
(B) REGISTRATION NUMBER: 30,946
(C) REFERENCE/DOCKET NUMBER: LUD 5356-PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (212) 688-9200
(B) TELEFAX: (212) 838-3884

(2) INFORMATION FOR SEQUENCE ID NO: 1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 462 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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ACCACAGGAG AATGAAAAGA ACCCGGGACT CCCAAAGACG CTAGATGTGT        50
GAAGATCCTG ATCACTCATT GGGTGTCTGA GTTCTGCGAT ATTCATCCCT       100
CAGCCAATGA GCTTACTGTT CTCGTGGGGG GTTTGTGAGC CTTGGGTAGG       150
AAGTTTTGCA AGTTCCGCCT ACAGCTCTAG CTTGTGAATT TGTACCCTTT       200
CACGTAAAAA AGTAGTCCAG AGTTTACTAC ACCCTCCCTC CCCCCTCCCA       250
CCTCGTGCTG TGCTGAGTTT AGAAGTCTTC CTTATAGAAG TCTTCCGTAT       300
AGAACTCTTC CGGAGGAAGG AGGGAGGACC CCCCCCCTTT GCTCTCCCAG       350
CATGCATTGT GTCAACGCCA TTGCACTGAG CTGGTCGAAG AAGTAAGCCG       400
CTAGCTTGCG ACTCTACTCT TATCTTAACT TAGCTCGGCT TCCTGCTGGT       450
ACCCTTTGTG CC                                               462
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(2) INFORMATION FOR SEQUENCE ID NO: 2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 675 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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ATG TCT GAT AAC AAG AAA CCA GAC AAA GCC CAC AGT GGC TCA GGT GGT    48
Met Ser Asp Asn Lys Lys Pro Asp Lys Ala His Ser Gly Ser Gly Gly
                5                  10                  15
GAC GGT GAT GGG AAT AGG TGC AAT TTA TTG CAC CGG TAC TCC CTG GAA    96
Asp Gly Asp Gly Asn Arg Cys Asn Leu Leu His Arg Tyr Ser Leu Glu
            20                  25                  30
GAA ATT CTG CCT TAT CTA GGG TGG CTG GTC TTC GCT GTT GTC ACA ACA   144
Glu Ile Leu Pro Tyr Leu Gly Trp Leu Val Phe Ala Val Val Thr Thr
        35                  40                  45
AGT TTT CTG GCG CTC CAG ATG TTC ATA GAC GCC CTT TAT GAG GAG CAG   192
Ser Phe Leu Ala Leu Gln Met Phe Ile Asp Ala Leu Tyr Glu Glu Gln
    50                  55                  60
TAT GAA AGG GAT GTG GCC TGG ATA GCC AGG CAA AGC AAG CGC ATG TCC   240
Tyr Glu Arg Asp Val Ala Trp Ile Ala Arg Gln Ser Lys Arg Met Ser
65                  70                  75                  80
TCT GTC GAT GAG GAT GAA GAC GAT GAG GAT GAT GAG GAT GAC TAC TAC   288
Ser Val Asp Glu Asp Glu Asp Asp Glu Asp Asp Glu Asp Asp Tyr Tyr
                85                  90                  95
GAC GAC GAG GAC GAC GAC GAC GAT GCC TTC TAT GAT GAT GAG GAT GAT   336
Asp Asp Glu Asp Asp Asp Asp Asp Ala Phe Tyr Asp Asp Glu Asp Asp
            100                 105                 110
GAG GAA GAA GAA TTG GAG AAC CTG ATG GAT GAT GAA TCA GAA GAT GAG   384
Glu Glu Glu Glu Leu Glu Asn Leu Met Asp Asp Glu Ser Glu Asp Glu
        115                 120                 125
GCC GAA GAA GAG ATG AGC GTG GAA ATG GGT GCC GGA GCT GAG GAA ATG   432
Ala Glu Glu Glu Met Ser Val Glu Met Gly Ala Gly Ala Glu Glu Met
    130                 135                 140
GGT GCT GGC GCT AAC TGT GCC TGT GTT CCT GGC CAT CAT TTA AGG AAG   480
Gly Ala Gly Ala Asn Cys Ala Cys Val Pro Gly His His Leu Arg Lys
145                 150                 155                 160
AAT GAA GTG AAG TGT AGG ATG ATT TAT TTC TTC CAC GAC CCT AAT TTC   528
Asn Glu Val Lys Cys Arg Met Ile Tyr Phe Phe His Asp Pro Asn Phe
                165                 170                 175
CTG GTG TCT ATA CCA GTG AAC CCT AAG GAA CAA ATG GAG TGT AGG TGT   576
Leu Val Ser Ile Pro Val Asn Pro Lys Glu Gln Met Glu Cys Arg Cys
            180                 185                 190
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BNSDOCID: <WO 9523874A1>

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GAA AAT GCT GAT GAA GAG GTT GCA ATG GAA GAG GAA GAA GAA GAA GAG   624
Glu Asn Ala Asp Glu Glu Val Ala Met Glu Glu Glu Glu Glu Glu Glu
        195                 200                 210
GAG GAG GAG GAG GAA GAG GAA ATG GGA AAC CCG GAT GGC TTC TCA CCT   672
Glu Glu Glu Glu Glu Glu Glu Met Gly Asn Pro Asp Gly Phe Ser Pro
220                 225                 230                 235
TAG                                                               675
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(2) INFORMATION FOR SEQUENCE ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 228 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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GCATGCAGTT GCAAAGCCCA GAAGAAAGAA ATGGACAGCG GAAGAAGTGG TTGTTTTTTT   60
TTCCCCTTCA TTAATTTTCT AGTTTTTAGT AATCCAGAAA ATTTGATTTT GTTCTAAAGT  120
TCATTATGCA AAGATGTCAC CAACAGACTT CTGACTGCAT GGTGAACTTT CATATGATAC  180
ATAGGATTAC ACTTGTACCT GTTAAAAATA AAAGTTTGAC TTGCATAC               228
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(2) INFORMATION FOR SEQUENCE ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1365 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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GAAGATCCTG ATCACTCATT GGGTGTCTGA GTTCTGCGAT ATTCATCCCT       100
CAGCCAATGA GCTTACTGTT CTCGTGGGGG GTTTGTGAGC CTTGGGTAGG       150
AAGTTTTGCA AGTTCCGCCT ACAGCTCTAG CTTGTGAATT TGTACCCTTT       200
CACGTAAAAA AGTAGTCCAG AGTTTACTAC ACCCTCCCTC CCCCCTCCCA       250
CCTCGTGCTG TGCTGAGTTT AGAAGTCTTC CTTATAGAAG TCTTCCGTAT       300
AGAACTCTTC CGGAGGAAGG AGGGAGGACC CCCCCCCTTT GCTCTCCCAG       350
CATGCATTGT GTCAACGCCA TTGCACTGAG CTGGTCGAAG AAGTAAGCCG       400
CTAGCTTGCG ACTCTACTCT TATCTTAACT TAGCTCGGCT TCCTGCTGGT       450
ACCCTTTGTG CC                                               462
ATG TCT GAT AAC AAG AAA CCA GAC AAA GCC CAC AGT GGC TCA      504
GGT GGT GAC GGT GAT GGG AAT AGG TGC AAT TTA TTG CAC CGG      546
TAC TCC CTG GAA GAA ATT CTG CCT TAT CTA GGG TGG CTG GTC      588
TTC GCT GTT GTC ACA ACA AGT TTT CTG GCG CTC CAG ATG TTC      630
ATA GAC GCC CTT TAT GAG GAG CAG TAT GAA AGG GAT GTG GCC      672
TGG ATA GCC AGG CAA AGC AAG CGC ATG TCC TCT GTC GAT GAG      714
GAT GAA GAC GAT GAG GAT GAT GAG GAT GAC TAC TAC GAC GAC      756
GAG GAC GAC GAC GAC GAT GCC TTC TAT GAT GAT GAG GAT GAT      798
GAG GAA GAA GAA TTG GAG AAC CTG ATG GAT GAT GAA TCA GAA      840
GAT GAG GCC GAA GAA GAG ATG AGC GTG GAA ATG GGT GCC GGA      882
GCT GAG GAA ATG GGT GCT GGC GCT AAC TGT GCC TGT GTT CCT      924
GGC CAT CAT TTA AGG AAG AAT GAA GTG AAG TGT AGG ATG ATT      966
TAT TTC TTC CAC GAC CCT AAT TTC CTG GTG TCT ATA CCA GTG     1008
AAC CCT AAG GAA CAA ATG GAG TGT AGG TGT GAA AAT GCT GAT     1050
GAA GAG GTT GCA ATG GAA GAG GAA GAA GAA GAA GAG GAG GAG     1092
GAG GAG GAA GAG GAA ATG GGA AAC CCG GAT GGC TTC TCA CCT     1134
TAG                                                        1137
GCATGCAGTT GCAAAGCCCA GAAGAAAGAA ATGGACAGCG GAAGAAGTGG      1187
TTGTTTTTTT TTCCCCTTCA TTAATTTTCT AGTTTTTAGT AATCCAGAAA      1237
ATTTGATTTT GTTCTAAAGT TCATTATGCA AAGATGTCAC CAACAGACTT      1287
CTGACTGCAT GGTGAACTTT CATATGATAC ATAGGATTAC ACTTGTACCT      1337
GTTAAAAATA AAAGTTTGAC TTGCATAC                              1365
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BNSDOCID: <WO 9523874A1>

(2) INFORMATION FOR SEQUENCE ID NO: 5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4698 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

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GAAGATCCTG ATCACTCATT GGGTGTCTGA GTTCTGCGAT ATTCATCCCT       100
CAGCCAATGA GCTTACTGTT CTCGTGGGGG GTTTGTGAGC CTTGGGTAGG       150
AAGTTTTGCA AGTTCCGCCT ACAGCTCTAG CTTGTGAATT TGTACCCTTT       200
CACGTAAAAA AGTAGTCCAG AGTTTACTAC ACCCTCCCTC CCCCCTCCCA       250
CCTCGTGCTG TGCTGAGTTT AGAAGTCTTC CTTATAGAAG TCTTCCGTAT       300
AGAACTCTTC CGGAGGAAGG AGGGAGGACC CCCCCCCTTT GCTCTCCCAG       350
CATGCATTGT GTCAACGCCA TTGCACTGAG CTGGTCGAAG AAGTAAGCCG       400
CTAGCTTGCG ACTCTACTCT TATCTTAACT TAGCTCGGCT TCCTGCTGGT       450
ACCCTTTGTG CC                                               462
ATG TCT GAT AAC AAG AAA CCA GAC AAA GCC CAC AGT GGC TCA      504
GGT GGT GAC GGT GAT GGG AAT AGG TGC AAT TTA TTG CAC CGG      546
TAC TCC CTG GAA GAA ATT CTG CCT TAT CTA GGG TGG CTG GTC      588
TTC GCT GTT GTC ACA ACA AGT TTT CTG GCG CTC CAG ATG TTC      630
ATA GAC GCC CTT TAT GAG GAG CAG TAT GAA AGG GAT GTG GCC      672
TGG ATA GCC AGG CAA AGC AAG CGC ATG TCC TCT GTC GAT GAG      714
GAT GAA GAC GAT GAG GAT GAT GAG GAT GAC TAC TAC GAC GAC      756
GAG GAC GAC GAC GAC GAT GCC TTC TAT GAT GAT GAG GAT GAT      798
GAG GAA GAA GAA TTG GAG AAC CTG ATG GAT GAT GAA TCA GAA      840
GAT GAG GCC GAA GAA GAG ATG AGC GTG GAA ATG GGT GCC GGA      882
GCT GAG GAA ATG GGT GCT GGC GCT AAC TGT GCC T                916
GTGAGTAACC CGTGGTCTTT ACTCTAGATT CAGGTGGGGT GCATTCTTTA      966
CTCTTGCCCA CATCTGTAGT AAAGACCACA TTTTGGTTGG GGGTCATTGC     1016
TGGAGCCATT CCTGGCTCTC CTGTCCACGC CTATCCCCGC TCCTCCCATC     1066
CCCCACTCCT TGCTCCGCTC TCTTTCCTTT TCCCACCTTG CCTCTGGAGC     1116
TTCAGTCCAT CCTGCTCTGC TCCCTTTCCC CTTTGCTCTC CTTGCTCCCC     1166
TCCCCCTCGG CTCAACTTTT CGTGCCTTCT GCTCTCTGAT CCCCACCCTC     1216
TTCAGGCTTC CCCATTTGCT CCTCTCCCGA AACCCTCCCC TTCCTGTTCC     1266
CCTTTTCGCG CCTTTTCTTT CCTGCTCCCC TCCCCCTCCC TATTTACCTT     1316
TCACCAGCTT TGCTCTCCCT GCTCCCCTCC CCCTTTTGCA CCTTTTCTTT     1366
TCCTGCTCCC CTCCCCCTCC CCTCCCTGTT TACCCTTCAC CGCTTTTCCT     1416
CTACCTGCTT CCCTCCCCCT TGCTGCTCCC TCCCTATTTG CATTTTCGGG     1466
TGCTCCTCCC TCCCCCTCCC CCTCCCTCCC TATTTGCATT TTCGGGTGCT     1516
CCTCCCTCCC CCTCCCCAGG CCTTTTTTTT TTTTTTTTTT TTTTTTTTTT     1566
TTGGTTTTTC GAGACAGGGT TTCTCTTTGT ATCCCTGGCT GTCCTGGCAC     1616
TCACTCTGTA GACCAGGCTG GCCTCAAACT CAGAAATCTG CCTGCCTCTG     1666
CCTCCCAAAT GCTGGGATTA AAGGCTTGCA CCAGGACTGC CCCAGTGCAG     1716
GCCTTTCTTT TTTCTCCTCT CTGGTCTCCC TAATCCCTTT TCTGCATGTT     1766
AACTCCCCTT TTGGCACCTT TCCTTTACAG GACCCCCTCC CCCTCCCTGT     1816
TTCCCTTCCG GCACCCTTCC TAGCCCTGCT CTGTTCCCTC TCCCTGCTCC     1866
CCTCCCCCTC TTTGCTCGAC TTTTAGCAGC CTTACCTCTC CCTGCTTTCT     1916
GCCCCGTTCC CCTTTTTTGT GCCTTTCCTC CTGGCTCCCC TCCACCTTCC     1966
AGCTCACCTT TTTGTTTGTT TGGTTGTTTG GTTGTTTGGT TTGCTTTTTT     2016
TTTTTTTTTT GCACCTTGTT TTCCAAGATC CCCCTCCCCC TCCGGCTTCC     2066
CCTCTGTGTG CCTTTCCTGT TCCCTCCCCC TCGCTGGCTC CCCCTCCCTT     2116
TCTGCCTTTC CTGTCCCTGC TCCCTTCTCT GCTAACCTTT TAATGCCTTT     2166
CTTTTCTAGA CTCCCCCCTC CAGGCTTGCT GTTTGCTTCT GTGCACTTTT     2216
CCTGACCCTG CTCCCCTTCC CCTCCCAGCT CCCCCCTCTT TTCCCACCTC     2266
CCTTTCTCCA GCCTGTCACC CCTCCTTCTC TCCTCTCTGT TTCTCCCACT     2316
TCCTGCTTCC TTTACCCCTT CCCTCTCCCT ACTCTCCTCC CTGCCTGCTG     2366
GACTTCCTCT CCAGCCGCCC AGTTCCCTGC AGTCCTGGAG TCTTTCCTGC     2416
CTCTCTGTCC ATCACTTCCC CCTAGTTTCA CTTCCCTTTC ACTCTCCCCT     2466
ATGTGTCTCT CTTCCTATCT ATCCCTTCCT TTCTGTCCCC TCTCCTCTGT     2516
CCATCACCTC TCTCCTCCCT TCCCTTTCCT CTCTCTTCCA TTTTCTTCCA     2566
CCTGCTTCTT TACCCTGCCT CTCCCATTGC CCTCTTACCT TTATGCCCAT     2616
TCCATGTCCC CTCTCAATTC CCTGTCCCAT TGTGCTCCCT CACATCTTCC     2666
ATTTCCCTCT TTCTCCCTTA GCCTCTTCTT CCTCTTCTCT TGTATCTCCC     2716
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BNSDOCID: <WO 9523874A1>

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TACTTGATCT TCTCTCCTCT CCACATACCC TTTTTCCTTT CCACCCTGCC      2816
CTTTGTCCCC AGACCCTACA GTATCCTGTG CACAGGAAGT GGGAGGTGCC      2866
ATCAACAACA AGGAGGCAAG AAACAGAGCA AAATCCCAAA ATCAGCAGGA      2916
AAGGCTGGAT GAAAATAAGG CCAGGTTCTG AGGACAGCTG GAATCTAGCC      2966
AAGTGGCTCC TATAACCCTA AGTACCAAGG GAGAAAGTGA TGGTGAAGTT      3016
CTTGATCCTT GCTGCTTCTT TTACATATGT TGGCACATCT TTCTCAAATG      3066
CAGGCCATGC TCCATGCTTG GCGCTTGCTC AGCGTGGTTA AGTAATGGGA      3116
GAATCTGAAA ACTAGGGGCC AGTGGTTTGT TTTGGGGACA AATTAGCACG      3166
TAGTGATATT TCCCCCTAAA AATTATAACA AACAGATTCA TGATTTGAGA      3216
TCCTTCTACA GGTGAGAAGT GGAAAAATTG TCACTATGAA GTTCTTTTTA      3266
GGCTAAAGAT ACTTGGAACC ATAGAAGCGT TGTTAAAATA CTGCTTTCTT      3316
TTGCTAAAAT ATTCTTTCTC ACATATTCAT ATTCTCCAG                  3355
 GT GTT CCT GGC CAT CAT TTA AGG AAG AAT GAA GTG AAG TGT      3396
AGG ATG ATT TAT TTC TTC CAC GAC CCT AAT TTC CTG GTG TCT      3438
ATA CCA GTG AAC CCT AAG GAA CAA ATG GAG TGT AGG TGT GAA      3480
AAT GCT GAT GAA GAG GTT GCA ATG GAA GAG GAA GAA GAA GAA      3522
GAG GAG GAG GAG GAG GAA GAG GAA ATG GGA AAC CCG GAT GGC      3564
TTC TCA CCT TAG                                             3576
GCATGCAGGT ACTGGCTTCA CTAACCAACC ATTCCTAACA TATGCCTGTA       3626
GCTAAGAGCA TCTTTTTAAA AAATATTATT GGTAAACTAA ACAATTGTTA       3676
TCTTTTTACA TTAATAAGTA TTAAATTAAT CCAGTATACA GTTTTAAGAA       3726
CCCTAAGTTA AACAGAAGTC AATGATGTCT AGATGCCTGT TCTTTAGATT       3776
GTAGTGAGAC TACTTACTAC AGATGAGAAG TTGTTAGACT CGGGAGTAGA       3826
GACCAGTAAA AGATCATGCA GTGAAATGTG GCCATGGAAA TCGCATATTG       3876
TTCTTATAGT ACCTTTGAGA CAGCTGATAA CAGCTGACAA AAATAAGTGT       3926
TTCAAGAAAG ATCACACGCC ATGGTTCACA TGCAAATTAT TATTTTGTCG       3976
TTCTGATTTT TTTCATTTCT AGACCTGTGG TTTTAAAGAG ATGAAAATCT       4026
CTTAAAATTT CCTTCATCTT TAATTTTCCT TAACTTTAGT TTTTTTCACT       4076
TAGAATTCAA TTCAAATTCT TAATTCAATC TTAATTTTTA GATTTCTTAA       4126
AATGTTTTTT AAAAAAAAATG CAAATCTCAT TTTTAAGAGA TGAAAGCAGA      4176
GTAACTGGGG GGCTTAGGGA ATCTGTAGGG TTGCGGTATA GCAATAGGGA       4226
GTTCTGGTCT CTGAGAAGCA GTCAGAGAGA ATGGAAAACC AGGCCCTTGC       4276
CAGTAGGTTA GTGAGGTTGA TATGATCAGA TTATGGACAC TCTCCAAATC       4326
ATAAATACTC TAACAGCTAA GGATCTCTGA GGGAAACACA ACAGGGAAAT       4376
ATTTTAGTTT CTCCTTGAGA AACAATGACA AGACATAAAA TTGGCAAGAA       4426
AGTCAGGAGT GTATTCTAAT AAGTGTTGCT TATCTCTTAT TTTCTTCTAC       4476
AGTTGCAAAG CCCAGAAGAA AGAAATGGAC AGCGGAAGAA GTGGTTGTTT       4526
TTTTTTCCCC TTCATTAATT TTCTAGTTTT TAGTAATCCA GAAAATTTGA       4576
TTTTGTTCTA AAGTTCATTA TGCAAAGATG TCACCAACAG ACTTCTGACT       4626
GCATGGTGAA CTTTCATATG ATACATAGGA TTACACTTGT ACCTGTTAAA       4676
AATAAAAGTT TGACTTGCAT AC                                    4698
```

(2) INFORMATION FOR SEQUENCE ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

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Leu Pro Tyr Leu Gly Trp Leu Val Phe
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(2) INFORMATION FOR SEQUENCE ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2418 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

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GGATCCAGGC CCTGCCAGGA AAAATATAAG GGCCCTGCGT GAGAACAGAG       50
GGGGTCATCC ACTGCATGAG AGTGGGGATG TCACAGAGTC CAGCCCACCC      100
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TCCTGGTAGC ACTGAGAAGC CAGGGCTGTG CTTGCGGTCT GCACCCTGAG      150
GGCCCGTGGA TTCCTCTTCC TGGAGCTCCA GGAACCAGGC AGTGAGGCCT      200
TGGTCTGAGA CAGTATCCTC AGGTCACAGA GCAGAGGATG CACAGGGTGT      250
GCCAGCAGTG AATGTTTGCC CTGAATGCAC ACCAAGGGCC CCACCTGCCA      300
CAGGACACAT AGGACTCCAC AGAGTCTGGC CTCACCTCCC TACTGTCAGT      350
CCTGTAGAAT CGACCTCTGC TGGCCGGCTG TACCCTGAGT ACCCTCTCAC      400
TTCCTCCTTC AGGTTTTCAG GGGACAGGCC AACCCAGAGG ACAGGATTCC      450
CTGGAGGCCA CAGAGGAGCA CCAAGGAGAA GATCTGTAAG TAGGCCTTTG      500
TTAGAGTCTC CAAGGTTCAG TTCTCAGCTG AGGCCTCTCA CACACTCCCT      550
CTCTCCCCAG GCCTGTGGGT CTTCATTGCC CAGCTCCTGC CCACACTCCT      600
GCCTGCTGCC CTGACGAGAG TCATCATGTC TCTTGAGCAG AGGAGTCTGC      650
ACTGCAAGCC TGAGGAAGCC CTTGAGGCCC AACAAGAGGC CCTGGGCCTG      700
GTGTGTGTGC AGGCTGCCAC CTCCTCCTCC TCTCCTCTGG TCCTGGGCAC      750
CCTGGAGGAG GTGCCCACTG CTGGGTCAAC AGATCCTCCC CAGAGTCCTC      800
AGGGAGCCTC CGCCTTTCCC ACTACCATCA ACTTCACTCG ACAGAGGCAA      850
CCCAGTGAGG GTTCCAGCAG CCGTGAAGAG GAGGGGCCAA GCACCTCTTG      900
TATCCTGGAG TCCTTGTTCC GAGCAGTAAT CACTAAGAAG GTGGCTGATT      950
TGGTTGGTTT TCTGCTCCTC AAATATCGAG CCAGGGAGCC AGTCACAAAG     1000
GCAGAAATGC TGGAGAGTGT CATCAAAAAT TACAAGCACT GTTTTCCTGA     1050
GATCTTCGGC AAAGCCTCTG AGTCCTTGCA GCTGGTCTTT GGCATTGACG     1100
TGAAGGAAGC AGACCCCACC GGCCACTCCT ATGTCCTTGT CACCTGCCTA     1150
GGTCTCTCCT ATGATGGCCT GCTGGGTGAT AATCAGATCA TGCCCAAGAC     1200
AGGCTTCCTG ATAATTGTCC TGGTCATGAT TGCAATGGAG GGCGGCCATG     1250
CTCCTGAGGA GGAAATCTGG GAGGAGCTGA GTGTGATGGA GGTGTATGAT     1300
GGGAGGGAGC ACAGTGCCTA TGGGGAGCCC AGGAAGCTGC TCACCCAAGA     1350
TTTGGTGCAG GAAAAGTACC TGGAGTACGG CAGGTGCCGG ACAGTGATCC     1400
CGCACGCTAT GAGTTCCTGT GGGGTCCAAG GGCCCTCGCT GAAACCAGCT     1450
ATGTGAAAGT CCTTGAGTAT GTGATCAAGG TCAGTGCAAG AGTTCGCTTT     1500
TTCTTCCCAT CCCTGCGTGA AGCAGCTTTG AGAGAGGAGG AAGAGGGAGT     1550
CTGAGCATGA GTTGCAGCCA AGGCCAGTGG GAGGGGGACT GGGCCAGTGC     1600
ACCTTCCAGG GCCGCGTCCA GCAGCTTCCC CTGCCTCGTG TGACATGAGG     1650
CCCATTCTTC ACTCTGAAGA GAGCGGTCAG TGTTCTCAGT AGTAGGTTTC     1700
TGTTCTATTG GGTGACTTGG AGATTTATCT TTGTTCTCTT TTGGAATTGT     1750
TCAAATGTTT TTTTTTAAGG GATGGTTGAA TGAACTTCAG CATCCAAGTT     1800
TATGAATGAC AGCAGTCACA CAGTTCTGTG TATATAGTTT AAGGGTAAGA     1850
GTCTTGTGTT TTATTCAGAT TGGGAAATCC ATTCTATTTT GTGAATTGGG     1900
ATAATAACAG CAGTGGAATA AGTACTTAGA AATGTGAAAA ATGAGCAGTA     1950
AAATAGATGA GATAAAGAAC TAAAGAAATT AAGAGATAGT CAATTCTTGC     2000
CTTATACCTC AGTCTATTCT GTAAAATTTT TAAAGATATA TGCATACCTG     2050
GATTTCCTTG GCTTCTTTGA GAATGTAAGA GAAATTAAAT CTGAATAAAG     2100
AATTCTTCCT GTTCACTGGC TCTTTTCTTC TCCATGCACT GAGCATCTGC     2150
TTTTTGGAAG GCCCTGGGTT AGTAGTGGAG ATGCTAAGGT AAGCCAGACT     2200
CATACCCACC CATAGGGTCG TAGAGTCTAG GAGCTGCAGT CACGTAATCG     2250
AGGTGGCAAG ATGTCCTCTA AAGATGTAGG GAAAAGTGAG AGAGGGGTGA     2300
GGGTGTGGGG CTCCGGGTGA GAGTGGTGGA GTGTCAATGC CCTGAGCTGG     2350
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AATGATCTTG GGTGGATCC                                      2418
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(2) INFORMATION FOR SEQUENCE ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5724 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-1 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

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CCCGGGGCAC CACTGGCATC CCTCCCCCTA CCACCCCCAA TCCCTCCCTT       50
TACGCCACCC ATCCAAACAT CTTCACGCTC ACCCCCAGCC CAAGCCAGGC      100
AGAATCCGGT TCCACCCCTG CTCTCAACCC AGGGAAGCCC AGGTGCCCAG      150
ATGTGACGCC ACTGACTTGA GCATTAGTGG TTAGAGAGAA GCGAGGTTTT      200
CGGTCTGAGG GGCGGCTTGA GATCGGTGGA GGGAAGCGGG CCCAGCTCTG      250
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AGATAGAGGA CCCCAAATAA TCCCTTCATG CCAGTCCTGG ACCATCTGGT      350
GGTGGACTTC TCAGGCTGGG CCACCCCCAG CCCCCTTGCT GCTTAAACCA      400
CTGGGGACTC GAAGTCAGAG CTCCGTGTGA TCAGGGAAGG GCTGCTTAGG      450
AGAGGGCAGC GTCCAGGCTC TGCCAGACAT CATGCTCAGG ATTCTCAAGG      500
AGGGCTGAGG GTCCCTAAGA CCCCACTCCC GTGACCCAAC CCCCACTCCA      550
ATGCTCACTC CCGTGACCCA ACCCCCTCTT CATTGTCATT CCAACCCCCA      600
CCCCACATCC CCCACCCCAT CCCTCAACCC TGATGCCCAT CCGCCCAGCC      650
ATTCCACCCT CACCCCCACC CCCACCCCCA CGCCCACTCC CACCCCCACC      700
CAGGCAGGAT CCGGTTCCCG CCAGGAAACA TCCGGGTGCC CGGATGTGAC      750
GCCACTGACT TGCGCATTGT GGGGCAGAGA GAAGCGAGGT TTCCATTCTG      800
AGGGACGGCG TAGAGTTCGG CCGAAGGAAC CTGACCCAGG CTCTGTGAGG      850
AGGCAAGGTG AGAGGCTGAG GGAGGACTGA GGACCCCGCC ACTCCAAATA      900
GAGAGCCCCA AATATTCCAG CCCCGCCCTT GCTGCCAGCC CTGGCCCACC      950
CGCGGGAAGA CGTCTCAGCC TGGGCTGCCC CCAGACCCCT GCTCCAAAAG     1000
CCTTGAGAGA CACCAGGTTC TTCTCCCCAA GCTCTGGAAT CAGAGGTTGC     1050
TGTGACCAGG GCAGGACTGG TTAGGAGAGG GCAGGGCACA GGCTCTGCCA     1100
GGCATCAAGA TCAGCACCCA AGAGGGAGGG CTGTGGGCCC CCAAGACTGC     1150
ACTCCAATCC CCACTCCCAC CCCATTCGCA TTCCCATTCC CCACCCAACC     1200
CCCATCTCCT CAGCTACACC TCCACCCCCA TCCCTACTCC TACTCCGTCA     1250
CCTGACCACC ACCCTCCAGC CCCAGCACCA GCCCCAACCC TTCTGCCACC     1300
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ATCCACTGAG GGGAGTGGTT TTAGGCTCTG TGAGGAGGCA AGGTGAGATG     1550
CTGAGGGAGG ACTGAGGAGG CACACACCCC AGGTAGATGG CCCCAAAATG     1600
ATCCAGTACC ACCCCTGCTG CCAGCCCTGG ACCACCCGGC CAGGACAGAT     1650
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GGCAATCTGT AGTCATAGCT TATGTGACCG GGGCAGGGTT GGTCAGGAGA     1750
GGCAGGGCCC AGGCATCAAG GTCCAGCATC CGCCCGGCAT TAGGGTCAGG     1800
ACCCTGGGAG GGAACTGAGG GTTCCCCACC CACACCTGTC TCCTCATCTC     1850
CACCGCCACC CCACTCACAT TCCCATACCT ACCCCCTACC CCCAACCTCA     1900
TCTTGTCAGA ATCCCTGCTG TCAACCCACG GAAGCCACGG GAATGGCGGC     1950
CAGGCACTCG GATCTTGACG TCCCCATCCA GGGTCTGATG GAGGGAAGGG     2000
GCTTGAACAG GGCCTCAGGG GAGCAGAGGG AGGGCCCTAC TGCGAGATGA     2050
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ACTGAGGCTG CCACTTCTGG CCTCAAGAAT CAGAACGATG GGGACTCAGA     2150
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ACGGGGAGGC CTTGGTCTGA GAAGGCTGCG CTCAGGTCAG TAGAGGGAGC     3050
GTCCCAGGCC CTGCCAGGAG TCAAGGTGAG GACCAAGCGG GCACCTCACC     3150
CAGGACACAT TAATTCCAAT GAATTTTGAT ATCTCTTGCT GCCCTTCCCC     3200
AAGGACCTAG GCACGTGTGG CCAGATGTTT GTCCCCTCCT GTCCTTCCAT     3250
TCCTTATCAT GGATGTGAAC TCTTGATTTG GATTTCTCAG ACCAGCAAAA     3300
GGGCAGGATC CAGGCCCTGC CAGGAAAAAT ATAAGGGCCC TGCGTGAGAA     3350
CAGAGGGGGT CATCCACTGC ATGAGAGTGG GGATGTCACA GAGTCCAGCC     3400
CACCCTCCTG GTAGCACTGA GAAGCCAGGG CTGTGCTTGC GGTCTGCACC     3450
CTGAGGGCCC GTGGATTCCT CTTCCTGGAG CTCCAGGAAC CAGGCAGTGA     3500
GGCCTTGGTC TGAGACAGTA TCCTCAGGTC ACAGAGCAGA GGATGCACAG     3550
GGTGTGCCAG CAGTGAATGT TTGCCCTGAA TGCACACCAA GGGCCCCACC     3600
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CTCACTTCCT CCTTCAGGTT TTCAGGGGAC AGGCCAACCC AGAGGACAGG      3750
ATTCCCTGGA GGCCACAGAG GAGCACCAAG GAGAAGATCT GTAAGTAGGC      3800
CTTTGTTAGA GTCTCCAAGG TTCAGTTCTC AGCTGAGGCC TCTCACACAC      3850
TCCCTCTCTC CCCAGGCCTG TGGGTCTTCA TTGCCCAGCT CCTGCCCACA      3900
CTCCTGCCTG CTGCCCTGAC GAGAGTCATC                            3930
ATG TCT CTT GAG CAG AGG AGT CTG CAC TGC AAG CCT GAG GAA      3972
GCC CTT GAG GCC CAA CAA GAG GCC CTG GGC CTG GTG TGT GTG      4014
CAG GCT GCC ACC TCC TCC TCT CCT CTG GTC CTG GGC ACC         4056
CTG GAG GAG GTG CCC ACT GCT GGG TCA ACA GAT CCT CCC CAG      4098
AGT CCT CAG GGA GCC TCC GCC TTT CCC ACT ACC ATC AAC TTC      4140
ACT CGA CAG AGG CAA CCC AGT GAG GGT TCC AGC AGC CGT GAA      4182
GAG GAG GGG CCA AGC ACC TCT TGT ATC CTG GAG TCC TTG TTC      4224
CGA GCA GTA ATC ACT AAG AAG GTG GCT GAT TTG GTT GGT TTT      4266
CTG CTC CTC AAA TAT CGA GCC AGG GAG CCA GTC ACA AAG GCA      4308
GAA ATG CTG GAG AGT GTC ATC AAA AAT TAC AAG CAC TGT TTT      4350
CCT GAG ATC TTC GGC AAA GCC TCT GAG TCC TTG CAG CTG GTC      4392
TTT GGC ATT GAC GTG AAG GAA GCA GAC CCC ACC GGC CAC TCC      4434
TAT GTC CTT GTC ACC TGC CTA GGT CTC TCC TAT GAT GGC CTG      4476
CTG GGT GAT AAT CAG ATC ATG CCC AAG ACA GGC TTC CTG ATA      4518
ATT GTC CTG GTC ATG ATT GCA ATG GAG GGC GGC CAT GCT CCT      4560
GAG GAG GAA ATC TGG GAG GAG CTG AGT GTG ATG GAG GTG TAT      4602
GAT GGG AGG GAG CAC AGT GCC TAT GGG GAG CCC AGG AAG CTG      4644
CTC ACC CAA GAT TTG GTG CAG GAA AAG TAC CTG GAG TAC GGC      4686
AGG TGC CGG ACA GTG ATC CCG CAC GCT ATG AGT TCC TGT GGG      4728
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AATCGAGGTG GCAAGATGTC CTCTAAAGAT GTAGGGAAAA GTGAGAGAGG      5600
GGTGAGGGTG TGGGGCTCCG GGTGAGAGTG GTGGAGTGTC AATGCCCTGA      5650
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ATTGTAATGA TCTTGGGTGG ATCC                                  5724
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(2) INFORMATION FOR SEQUENCE ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4157 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-2 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

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GGTCAGAGGA CAGCGAGATT CTCGCCCTGA GCAACGGCCT GACGTCGGCG       150
GAGGGAAGCA GGCGCAGGCT CCGTGAGGAG GCAAGGTAAG ACGCCGAGGG       200
AGGACTGAGG CGGGCCTCAC CCCAGACAGA GGGCCCCCAA TTAATCCAGC       250
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GCTGCCTCTG CTGCCGGGCC TGGACCACCC TGCAGGGGAA GACTTCTCAG      300
GCTCAGTCGC CACCACCTCA CCCCGCCACC CCCCGCCGCT TTAACCGCAG      350
GGAACTCTGG CGTAAGAGCT TTGTGTGACC AGGGCAGGGC TGGTTAGAAG      400
TGCTCAGGGC CCAGACTCAG CCAGGAATCA AGGTCAGGAC CCCAAGAGGG      450
GACTGAGGGC AACCCACCCC CTACCCTCAC TACCAATCCC ATCCCCCAAC      500
ACCAACCCCA CCCCCATCCC TCAAACACCA ACCCCACCCC CAAACCCCAT      550
TCCCATCTCC TCCCCCACCA CCATCCTGGC AGAATCCGGC TTTGCCCCTG      600
CAATCAACCC ACGGAAGCTC CGGGAATGGC GGCCAAGCAC GCGGATCCTG      650
ACGTTCACAT GTACGGCTAA GGGAGGGAAG GGGTTGGGTC TCGTGAGTAT      700
GGCCTTTGGG ATGCAGAGGA AGGGCCCAGG CCTCCTGGAA GACAGTGGAG      750
TCCTTAGGGG ACCCAGCATG CCAGGACAGG GGGCCCACTG TACCCCTGTC      800
TCAAACTGAG CCACCTTTTC ATTCAGCCGA GGGAATCCTA GGGATGCAGA      850
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ACAGGGGCCC CTCTGGTCGA CAGATGCAGT GGTTCTAGGA TCTGCCAAGC     1550
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GCAGCAAGGG GGCCCCATAG AAATCTGCCC TGCCCCTGCG GTTACTTCAG     1650
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TCT ACT CTA GTG GAA GTT ACC CTG GGG GAG GTG CCT GCT GCC    2765
GAC TCA CCG AGT CCT CCC CAC AGT CCT CAG GGA GCC TCC AGC    2807
TTC TCG ACT ACC ATC AAC TAC ACT CTT TGG AGA CAA TCC GAT    2849
GAG GGC TCC AGC AAC CAA GAA GAG GAG GGG CCA AGA ATG TTT    2891
CCC GAC CTG GAG TCC GAG TTC CAA GCA GCA ATC AGT AGG AAG    2933
ATG GTT GAG TTG GTT CAT TTT CTG CTC CTC AAG TAT CGA GCC    2975
AGG GAG CCG GTC ACA AAG GCA GAA ATG CTG GAG AGT GTC CTC    3017
AGA AAT TGC CAG GAC TTC TTT CCC GTG ATC TTC AGC AAA GCC    3059
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GTG GTC CCC ATC AGC CAC TTG TAC ATC CTT GTC ACC TGC CTG    3143
GGC CTC TCC TAC GAT GGC CTG CTG GGC GAC AAT CAG GTC ATG    3185
CCC AAG ACA GGC CTC CTG ATA ATC GTC CTG GCC ATA ATC GCA    3227
ATA GAG GGC GAC TGT GCC CCT GAG GAG AAA ATC TGG GAG GAG    3269
CTG AGT ATG TTG GAG GTG TTT GAG GGG AGG GAG GAC AGT GTC    3311
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GAA AAC TAC CTG GAG TAC CGG CAG GTG CCC GGC AGT GAT CCT    3395
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TGCCTTATAC CTCAGTCTAT TATGTAAAAT TAAAAATATG TGTATGTTTT      4042
TGCTTCTTTG AGAATGCAAA AGAAATTAAA TCTGAATAAA TTCTTCCTGT      4092
TCACTGGCTC ATTTCTTTAC CATTCACTCA GCATCTGCTC TGTGGAAGGC      4142
CCTGGTAGTA GTGGG                                           4157
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(2) INFORMATION FOR SEQUENCE ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 662 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-21 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

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CAGAGAACAG CGAGATTCTC GCCCTGAGCA ACGGCCTGAC GTCGGCGGAG      150
GGAAGCAGGC GCAGGCTCCG TGAGGAGGCA AGGTAAGATG CCGAGGGAGG      200
ACTGAGGCGG GCCTCACCCC AGACAGAGGG CCCCCAATAA TCCAGCGCTG      250
CCTCTGCTGC CAGGCCTGGA CCACCCTGCA GGGGAAGACT TCTCAGGCTC      300
AGTCGCCACC ACCTCACCCC GCCACCCCCC GCCGCTTTAA CCGCAGGGAA      350
CTCTGGTGTA AGAGCTTTGT GTGACCAGGG CAGGGCTGGT TAGAAGTGCT      400
CAGGGCCCAG ACTCAGCCAG GAATCAAGGT CAGGACCCCA AGAGGGGACT      450
GAGGGTAACC CCCCCGCACC CCCACCACCA TTCCCATCCC CCAACACCAA      500
CCCCACCCCC ATCCCCCAAC ACCAAACCCA CCACCATCGC TCAAACATCA      550
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GAGCTTTGCC CCTGCAATCA ACCCACGGAA GCTCCGGGAA TGGCGGCCAA      650
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(2) INFORMATION FOR SEQUENCE ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1640 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(ix) FEATURE:

(A) NAME/KEY: cDNA MAGE-3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

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GTTGCCCTGA CCAGAGTCAT C                                     171
ATG CCT CTT GAG CAG AGG AGT CAG CAC TGC AAG CCT GAA GAA      213
GGC CTT GAG GCC CGA GGA GAG GCC CTG GGC CTG GTG GGT GCG      255
CAG GCT CCT GCT ACT GAG GAG CAG GAG GCT GCC TCC TCC TCT      297
TCT ACT CTA GTT GAA GTC ACC CTG GGG GAG GTG CCT GCT GCC      339
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CTC CCC ACT ACC ATG AAC TAC CCT CTC TGG AGC CAA TCC TAT      423
GAG GAC TCC AGC AAC CAA GAA GAG GAG GGG CCA AGC ACC TTC      465
CCT GAC CTG GAG TCC GAG TTC CAA GCA GCA CTC AGT AGG AAG      507
GTG GCC GAG TTG GTT CAT TTT CTG CTC CTC AAG TAT CGA GCC      549
AGG GAG CCG GTC ACA AAG GCA GAA ATG CTG GGG AGT GTC GTC      591
GGA AAT TGG CAG TAT TTC TTT CCT GTG ATC TTC AGC AAA GCT      633
TCC AGT TCC TTG CAG CTG GTC TTT GGC ATC GAG CTG ATG GAA      675
GTG GAC CCC ATC GGC CAC TTG TAC ATC TTT GCC ACC TGC CTG      717
GGC CTC TCC TAC GAT GGC CTG CTG GGT GAC AAT CAG ATC ATG      759
CCC AAG GCA GGC CTC CTG ATA ATC GTC CTG GCC ATA ATC GCA      801
AGA GAG GGC GAC TGT GCC CCT GAG GAG AAA ATC TGG GAG GAG      843
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TTG GGG GAT CCC AAG AAG CTG CTC ACC CAA CAT TTC GTG CAG      927
GAA AAC TAC CTG GAG TAC CGG CAG GTC CCC GGC AGT GAT CCT      969
GCA TGT TAT GAA TTC CTG TGG GGT CCA AGG GCC CTC GTT GAA     1011
ACC AGC TAT GTG AAA GTC CTG CAC CAT ATG GTA AAG ATC AGT     1053
GGA GGA CCT CAC ATT TCC TAC CCA CCC CTG CAT GAG TGG GTT     1095
TTG AGA GAG GGG GAA GAG TGA                                1116
GTCTGAGCAC GAGTTGCAGC CAGGGCCAGT GGGAGGGGGT CTGGGCCAGT     1166
GCACCTTCCG GGGCCGCATC CCTTAGTTTC CACTGCCTCC TGTGACGTGA     1216
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TAAGAGTCTT GttTTTTACT CAAATTgGGA AATCCATTCC ATTTTGTGAA     1466
TTGTGACATA ATAATAGCAG TGGTAAAAGT ATTTGCTTAA AATTGTGAGC     1516
GAATTAGCAA TAACATACAT GAGATAACTC AAGAAATCAA AAGATAGTTG     1566
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(2) INFORMATION FOR SEQUENCE ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 943 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-31 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

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GCCCGTGGAT TCCTCTCCCA GGAATCAGGA GCTCCAGGAA CAAGGCAGTG      150
AGGACTTGGT CTGAGGCAGT GTCCTCAGGT CACAGAGTAG AGGGGgCTCA      200
GATAGTGCCA ACGGTGAAGG TTTGCCTTGG ATTCAAACCA AGGGCCCCAC      250
CTGCCCCAGA ACACATGGAC TCCAGAGCGC CTGGCCTCAC CCTCAATACT      300
TTCAGTCCTG CAGCCTCAGC ATGCGCTGGC CGGATGTACC CTGAGGTGCC      350
CTCTCACTTC CTCCTTCAGG TTCTGAGGGG ACAGGCTGAC CTGGAGGACC      400
AGAGGCCCCC GGAGGAGCAC TGAAGGAGAA GATCTGTAAG TAAGCCTTTG      450
TTAGAGCCTC CAAGGTTCCA TTCAGTACTC AGCTGAGGTC TCTCACATGC      500
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CTCCCGCCTG TTGCCCTGAC CAGAGTCATC                            580
ATG CCT CTT GAG CAG AGG AGT CAG CAC TGC AAG CCT GAA GAA      622
GGC CTT GAG GCC CGA GGA GAg GCC CTG GGC CTG GTG GGT GCG      664
CAG GCT CCT GCT ACT GAG GAG CAG GAG GCT GCC TCC TCC TCT      706
TCT AGT GTA GTT GAA GTC ACC CTG GGG GAG GTG CCT GCT GCC      748
GAG TCA CCA GAT CCT CCC CAG AGT CCT CAG GGA GCC TCC AGC      790
CTC CCC ACT ACC ATG AAC TAC CCT CTC TGG AGC CAA TCC TAT      832
GAG GAC TCC AGC AAC CAA GAA GAG GAG GGG CCA AGC ACC TTC      874
CCT GAC CTG GAG TCT GAG TTC CAA GCA GCA CTC AGT AGG AAG      916
GTG GCC AAG TTG GTT CAT TTT CTG CTC                         943
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BNSDOCID: <WO 9523874A1>

(2) INFORMATION FOR SEQUENCE ID NO: 13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2531 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-4 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GGATCCAGGC CCTGCCTGGA GAAATGTGAG GGCCCTGAGT GAACACAGTG 50
GGGATCATCC ACTCCATGAG AGTGGGGACC TCACAGAGTC CAGCCTACCC 100
TCTTGATGGC ACTGAGGGAC CGGGGCTGTG CTTACAGTCT GCACCCTAAG 150
GGCCCATGGA TTCCTCTCCT AGGAGCTCCA GGAACAAGGC AGTGAGGCCT 200
TGGTCTGAGA CAGTGTCCTC AGGTTACAGA GCAGAGGATG CACAGGCTGT 250
GCCAGCAGTG AATGTTTGCC CTGAATGCAC ACCAAGGGCC CCACCTGCCA 300
CAAGACACAT AGGACTCCAA AGAGTCTGGC CTCACCTCCC TACCATCAAT 350
CCTGCAGAAT CGACCTCTGC TGGCCGGCTA TACCCTGAGG TGCTCTCTCA 400
CTTCCTCCTT CAGGTTCTGA GCAGACAGGC CAACCGGAGA CAGGATTCCC 450
TGGAGGCCAC AGAGGAGCAC CAAGGAGAAG ATCTGTAAGT AAGCCTTTGT 500
TAGAGCCTCT AAGATTTGGT TCTCAGCTGA GGTCTCTCAC ATGCTCCCTC 550
TCTCCGTAGG CCTGTGGGTC CCCATTGCCC AGCTTTTGCC TGCACTCTTG 600
CCTGCTGCCC TGACCAGAGT CATC 624
ATG TCT TCT GAG CAG AAG AGT CAG CAC TGC AAG CCT GAG GAA 666
GGC GTT GAG GCC CAA GAA GAG GCC CTG GGC CTG GTG GGT GCA 708
CAG GCT CCT ACT ACT GAG GAG CAG GAG GCT GCT GTC TCC TCC 750
TCC TCT CCT CTG GTC CCT GGC ACC CTG GAG GAA GTG CCT GCT 792
GCT GAG TCA GCA GGT CCT CCC CAG AGT CCT CAG GGA GCC TCT 834
GCC TTA CCC ACT ACC ATC AGC TTC ACT TGC TGG AGG CAA CCC 876
AAT GAG GGT TCC AGC AGC CAA GAA GAG GAG GGG CCA AGC ACC 918
TCG CCT GAC GCA GAG TCC TTG TTC CGA GAA GCA CTC AGT AAC 960
AAG GTG GAT GAG TTG GCT CAT TTT CTG CTC CGC AAG TAT CGA 1002
GCC AAG GAG CTG GTC ACA AAG GCA GAA ATG CTG GAG AGA GTC 1044
ATC AAA AAT TAC AAG CGC TGC TTT CCT GTG ATC TTC GGC AAA 1086
GCC TCC GAG TCC CTG AAG ATG ATC TTT GGC ATT GAC GTG AAG 1128
GAA GTG GAC CCC GCC AGC AAC ACC TAC ACC CTT GTC ACC TGC 1170
CTG GGC CTT TCC TAT GAT GGC CTG CTG GGT AAT AAT CAG ATC 1212
TTT CCC AAG ACA GGC CTT CTG ATA ATC GTC CTG GGC ACA ATT 1254
GCA ATG GAG GGC GAC AGC GCC TCT GAG GAG GAA ATC TGG GAG 1296
GAG CTG GGT GTG ATG GGG GTG TAT GAT GGG AGG GAG CAC ACT 1338
GTC TAT GGG GAG CCC AGG AAA CTG CTC ACC CAA GAT TGG GTG 1380
CAG GAA AAC TAC CTG GAG TAC CGG CAG GTA CCC GGC AGT AAT 1422
CCT GCG CGC TAT GAG TTC CTG TGG GGT CCA AGG GCT CTG GCT 1464
GAA ACC AGC TAT GTG AAA GTC CTG GAG CAT GTG GTC AGG GTC 1506
AAT GCA AGA GTT CGC ATT GCC TAC CCA TCC CTG CGT GAA GCA 1548
GCT TTG TTA GAG GAG GAA GAG GGA GTC TGA 1578
GCATGAGTTG CAGCCAGGGC TGTGGGGAAG GGGCAGGGCT GGGCCAGTGC 1628
ATCTAACAGC CCTGTGCAGC AGCTTCCCTT GCCTCGTGTA ACATGAGGCC 1678
CATTCTTCAC TCTGTTTGAA GAAAATAGTC AGTGTTCTTA GTAGTGGGTT 1728
TCTATTTTGT TGGATGACTT GGAGATTTAT CTCTGTTTCC TTTTACAATT 1778
GTTGAAATGT TCCTTTTAAT GGATGGTTGA ATTAACTTCA GCATCCAAGT 1828
TTATGAATCG TAGTTAACGT ATATTGCTGT TAATATAGTT TAGGAGTAAG 1878
AGTCTTGTTT TTTATTCAGA TTGGGAAATC CGTTCTATTT TGTGAATTTG 1928
GGACATAATA ACAGCAGTGG AGTAAGTATT TAGAAGTGTG AATTCACCGT 1978
GAAATAGGTG AGATAAATTA AAAGATACTT AATTCCCGCC TTATGCCTCA 2028
GTCTATTCTG TAAAATTTAA AAATATATAT GCATACCTGG ATTTCCTTGG 2078
CTTCGTGAAT GTAAGAGAAA TTAAATCTGA ATAAATAATT CTTTCTGTTA 2128
ACTGGCTCAT TTCTTCTCTA TGCACTGAGC ATCTGCTCTG TGGAAGGCCC 2178
AGGATTAGTA GTGGAGATAC TAGGGTAAGC CAGACACACA CCTACCGATA 2228
GGGTATTAAG AGTCTAGGAG CGCGGTCATA TAATTAAGGT GACAAGATGT 2278
CCTCTAAGAT GTAGGGGAAA AGTAACGAGT GTGGGTATGG GGCTCCAGGT 2328
GAGAGTGGTC GGGTGTAAAT TCCCTGTGTG GGGCCTTTTG GGCTTTGGGA 2378
AACTGCATTT TCTTCTGAGG GATCTGATTC TAATGAAGCT TGGTGGGTCC 2428
AGGGCCAGAT TCTCAGAGGG AGAGGGAAAA GCCCAGATTG GAAAAGTTGC 2478

TCTGAGCAGT TCCTTTGTGA CAATGGATGA ACAGAGAGGA GCCTCTACCT 2528
GGG 2531

(2) INFORMATION FOR SEQUENCE ID NO: 14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2531 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-41 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GGATCCAGGC CCTGCCTGGA GAAATGTGAG GGCCCTGAGT GAACACAGTG 50
GGGATCATCC ACTCCATGAG AGTGGGGACC TCACAGAGTC CAGCCTACCC 100
TCTTGATGGC ACTGAGGGAC CGGGGCTGTG CTTACAGTCT GCACCCTAAG 150
GGCCCATGGA TTCCTCTCCT AGGAGCTCCA GGAACAAGGC AGTGAGGCCT 200
TGGTCTGAGA CAGTGTCCTC AGGTTACAGA GCAGAGGATG CACAGGCTGT 250
GCCAGCAGTG AATGTTTGCC CTGAATGCAC ACCAAGGGCC CCACCTGCCA 300
CAAGACACAT AGGACTCCAA AGAGTCTGGC CTCACCTCCC TACCATCAAT 350
CCTGCAGAAT CGACCTCTGC TGGCCGGCTA TACCCTGAGG TGCTCTCTCA 400
CTTCCTCCTT CAGGTTCTGA GCAGACAGGC CAACCGGAGA CAGGATTCCC 450
TGGAGGCCAC AGAGGAGCAC CAAGGAGAAG ATCTGTAAGT AAGCCTTTGT 500
TAGAGCCTCT AAGATTTGGT TCTCAGCTGA GGTCTCTCAC ATGCTCCCTC 550
TCTCCGTAGG CCTGTGGGTC CCCATTGCCC AGCTTTTGCC TGCACTCTTG 600
CCTGCTGCCC TGAGCAGAGT CATC 624
ATG TCT TCT GAG CAG AAG AGT CAG CAC TGC AAG CCT GAG GAA 666
GGC GTT GAG GCC CAA GAA GAG GCC CTG GGC CTG GTG GGT GCG 708
CAG GCT CCT ACT ACT GAG GAG CAG GAG GCT GCT GTC TCC TCC 750
TCC TCT CCT CTG GTC CCT GGC ACC CTG GAG GAA GTG CCT GCT 792
GCT GAG TCA GCA GGT CCT CCC CAG AGT CCT CAG GGA GCC TCT 834
GCC TTA CCC ACT ACC ATC AGC TTC ACT TGC TGG AGG CAA CCC 876
AAT GAG GGT TCC AGC AGC CAA GAA GAG GAG GGG CCA AGC ACC 918
TCG CCT GAC GCA GAG TCC TTG TTC CGA GAA GCA CTC AGT AAC 960
AAG GTG GAT GAG TTG GCT CAT TTT CTG CTC CGC AAG TAT CGA 1002
GCC AAG GAG CTG GTC ACA AAG GCA GAA ATG CTG GAG AGA GTC 1044
ATC AAA AAT TAC AAG CGC TGC TTT CCT GTG ATC TTC GGC AAA 1086
GCC TCC GAG TCC CTG AAG ATG ATC TTT GGC ATT GAC GTG AAG 1128
GAA GTG GAC CCC ACC AGC AAC ACC TAC ACC CTT GTC ACC TGC 1170
CTG GGC CTT TCC TAT GAT GGC CTG CTG GGT AAT AAT CAG ATC 1212
TTT CCC AAG ACA GGC CTT CTG ATA ATC GTC CTG GGC ACA ATT 1254
GCA ATG GAG GGC GAC AGC GCC TCT GAG GAG GAA ATC TGG GAG 1296
GAG CTG GGT GTG ATG GGG GTG TAT GAT GGG AGG GAG CAC ACT 1338
GTC TAT GGG GAG CCC AGG AAA CTG CTC ACC CAA GAT TGG GTG 1380
CAG GAA AAC TAC CTG GAG TAC CGG CAG GTA CCC GGC AGT AAT 1422
CCT GCG CGC TAT GAG TTC CTG TGG GGT CCA AGG GCT CTG GCT 1464
GAA ACC AGC TAT GTG AAA GTC CTG GAG CAT GTG GTC AGG GTC 1506
AAT GCA AGA GTT CGC ATT GCC TAC CCA TCC CTG CGT GAA GCA 1548
GCT TTG TTA GAG GAG GAA GAG GGA GTC TGA 1578
GCATGAGTTG CAGCCAGGGC TGTGGGGAAG GGGCAGGGCT GGGCCAGTGC 1628
ATCTAACAGC CCTGTGCAGC AGCTTCCCTT GCCTCGTGTA ACATGAGGCC 1678
CATTCTTCAC TCTGTTTGAA GAAAATAGTC AGTGTTCTTA GTAGTGGGTT 1728
TCTATTTTGT TGGATGACTT GGAGATTTAT CTCTGTTTCC TTTTACAATT 1778
GTTGAAATGT TCCTTTTAAT GGATGGTTGA ATTAACTTCA GCATCCAAGT 1828
TTATGAATCG TAGTTAACGT ATATTGCTGT TAATATAGTT TAGGAGTAAG 1878
AGTCTTGTTT TTTATTCAGA TTGGGAAATC CGTTCTATTT TGTGAATTTG 1928
GGACATAATA ACAGCAGTGG AGTAAGTATT TAGAAGTGTG AATTCACCGT 1978
GAAATAGGTG AGATAAATTA AAAGATACTT AATTCCCGCC TTATGCCTCA 2028
GTCTATTCTG TAAAATTTAA AAATATATAT GCATACCTGG ATTTCCTTGG 2078
CTTCGTGAAT GTAAGAGAAA TTAAATCTGA ATAAATAATT CTTTCTGTTA 2128
ACTGGCTCAT TTCTTCTCTA TGCACTGAGC ATCTGCTCTG TGGAAGGCCC 2178
AGGATTAGTA GTGGAGATAC TAGGGTAAGC CAGACACACA CCTACCGATA 2228

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GGGTATTAAG AGTCTAGGAG CGCGGTCATA TAATTAAGGT GACAAGATGT      2278
CCTCTAAGAT GTAGGGGAAA AGTAACGAGT GTGGGTATGG GGCTCCAGGT      2328
GAGAGTGGTC GGGTGTAAAT TCCCTGTGTG GGGCCTTTTG GGCTTTGGGA      2378
AACTCCATTT TCTTCTGAGG GATCTGATTC TAATGAAGCT TGGTGGGTCC      2428
AGGGCCAGAT TCTCAGAGGG AGAGGGAAAA GCCCAGATTG GAAAAGTTGC      2478
TCTGAGCGGT TCCTTTGTGA CAATGGATGA ACAGAGAGGA GCCTCTACCT      2528
GGG                                                        2531
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(2) INFORMATION FOR SEQUENCE ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1068 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(ix) FEATURE:

(A) NAME/KEY: cDNA MAGE-4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

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  G GGG CCA AGC ACC TCG CCT GAC GCA GAG TCC TTG TTC CGA       40
GAA GCA CTC AGT AAC AAG GTG GAT GAG TTG GCT CAT TTT CTG       82
CTC CGC AAG TAT CGA GCC AAG GAG CTG GTC ACA AAG GCA GAA      124
ATG CTG GAG AGA GTC ATC AAA AAT TAC AAG CGC TGC TTT CCT      166
GTG ATC TTC GGC AAA GCC TCC GAG TCC CTG AAG ATG ATC TTT      208
GGC ATT GAC GTG AAG GAA GTG GAC CCC GCC AGC AAC ACC TAC      250
ACC CTT GTC ACC TGC CTG GGC CTT TCC TAT GAT GGC CTG CTG      292
GGT AAT AAT CAG ATC TTT CCC AAG ACA GGC CTT CTG ATA ATC      334
GTC CTG GGC ACA ATT GCA ATG GAG GGC GAC AGC GCC TCT GAG      376
GAG GAA ATC TGG GAG GAG CTG GGT GTG ATG GGG GTG TAT GAT      418
GGG AGG GAG CAC ACT GTC TAT GGG GAG CCC AGG AAA CTG CTC      460
ACC CAA GAT TGG GTG CAG GAA AAC TAC CTG GAG TAC CGG CAG      502
GTA CCC GGC AGT AAT CCT GCG CGC TAT GAG TTC CTG TGG GGT      544
CCA AGG GCT CTG GCT GAA ACC AGC TAT GTG AAA GTC CTG GAG      586
CAT GTG GTC AGG GTC AAT GCA AGA GTT CGC ATT GCC TAC CCA      628
TCC CTG CGT GAA GCA GCT TTG TTA GAG GAG GAA GAG GGA GTC      670
TGAGCATGAG TTGCAGCCAG GGCTGTGGGG AAGGGGCAGG GCTGGGCCAG      720
TGCATCTAAC AGCCCTGTGC AGCAGCTTCC CTTGCCTCGT GTAACATGAG      770
GCCCATTCTT CACTCTGTTT GAAGAAAATA GTCAGTGTTC TTAGTAGTGG      820
GTTTCTATTT TGTTGGATGA CTTGGAGATT TATCTCTGTT TCCTTTTACA      870
ATTGTTGAAA TGTTCCTTTT AATGGATGGT TGAATTAACT TCAGCATCCA      920
AGTTTATGAA TCGTAGTTAA CGTATATTGC TGTTAATATA GTTTAGGAGT      970
AAGAGTCTTG TTTTTTATTC AGATTGGGAA ATCCGTTCTA TTTTGTGAAT     1020
TTGGGACATA ATAACAGCAG TGGAGTAAGT ATTTAGAAGT GTGAATTC       1068
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(2) INFORMATION FOR SEQUENCE ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2226 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-5 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

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GGATCCAGGC CTTGCCAGGA GAAAGGTGAG GGCCCTGTGT GAGCACAGAG       50
GGGACCATTC ACCCCAAGAG GGTGGAGACC TCACAGATTC CAGCCTACCC      100
TCCTGTTAGC ACTGGGGGCC TGAGGCTGTG CTTGCAGTCT GCACCCTGAG      150
GGCCCATGCA TTCCTCTTCC AGGAGCTCCA GGAAACAGAC ACTGAGGCCT      200
TGGTCTGAGG CCGTGCCCTC AGGTCACAGA GCAGAGGAGA TGCAGACGTC      250
TAGTGCCAGC AGTGAACGTT TGCCTTGAAT GCACACTAAT GGCCCCCATC      300
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BNSDOCID: <WO 9523874A1>

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GCCCCAGAAC ATATGGGACT CCAGAGCACC TGGCCTCACC CTCTCTACTG      350
TCAGTCCTGC AGAATCAGCC TCTGCTTGCT TGTGTACCCT GAGGTGCCCT      400
CTCACTTTTT CCTTCAGGTT CTCAGGGGAC AGGCTGACCA GGATCACCAG      450
GAAGCTCCAG AGGATCCCCA GGAGGCCCTA GAGGAGCACC AAAGGAGAAG      500
ATCTGTAAGT AAGCCTTTGT TAGAGCCTCC AAGGTTCAGT TTTTAGCTGA      550
GGCTTCTCAC ATGCTCCCTC TCTCTCCAGG CCAGTGGGTC TCCATTGCCC      600
AGCTCCTGCC CACACTCCTG CCTGTTGCGG TGACCAGAGT CGTC            644
ATG TCT CTT GAG CAG AAG AGT CAG CAC TGC AAG CCT GAG GAA      684
CTC CTC TGG TCC CAG GCA CCC TGG GGG AGG TGC CTG CTG CTG      728
GGT CAC CAG GTC CTC TCA AGA GTC CTC AGG GAG CCT CCG CCA      770
TCC CCA CTG CCA TCG ATT TCA CTC TAT GGA GGC AAT CCA TTA      812
AGG GCT CCA GCA ACC AAG AAG AGG AGG GGC CAA GCA CCT CCC      854
CTG ACC CAG AGT CTG TGT TCC GAG CAG CAC TCA GTA AGA AGG      896
TGG CTG ACT TGA                                            908
TTCATTTTCT GCTCCTCAAG TATTAAGTCA AGGAGCTGGT CACAAAGGCA      958
GAAATGCTGG AGAGCGTCAT CAAAAATTAC AAGCGCTGCT TTCCTGAGAT     1008
CTTCGGCAAA GCCTCCGAGT CCTTGCAGCT GGTCTTTGGC ATTGACGTGA     1058
AGGAAGCGGA CCCCACCAGC AACACCTACA CCCTTGTCAC CTGCCTGGGA     1108
CTCCTATGAT GGCCTGCTGG TTGATAATAA TCAGATCATG CCCAAGACGG     1158
GCCTCCTGAT AATCGTCTTG GGCATGATTG CAATGGAGGG CAAATGCGTC     1208
CCTGAGGAGA AAATCTGGGA GGAGCTGAGT GTGATGAAGG TGTATGTTGG     1258
GAGGGAGCAC AGTGTCTGTG GGGAGCCCAG GAAGCTGCTC ACCCAAGATT     1308
TGGTGCAGGA AAACTACCTG GAGTACCGGC AGGTGCCCAG CAGTGATCCC     1358
ATATGCTATG AGTTACTGTG GGGTCCAAGG GCACTCGCTG CTTGAAAGTA     1408
CTGGAGCACG TGGTCAGGGT CAATGCAAGA GTTCTCATTT CCTACCCATC     1458
CCTGCGTGAA GCAGCTTTGA GAGAGGAGGA AGAGGGAGTC TGAGCATGAG     1508
CTGCAGCCAG GGCCACTGCG AGGGGGGCTG GGCCAGTGCA CCTTCCAGGG     1558
CTCCGTCCAG TAGTTTCCCC TGCCTTAATG TGACATGAGG CCCATTCTTC     1608
TCTCTTTGAA GAGAGCAGTC AACATTCTTA GTAGTGGGTT TCTGTTCTAT     1658
TGGATGACTT TGAGATTTGT CTTTGTTTCC TTTTGGAATT GTTCAAATGT     1708
TTCTTTTAAT GGGTGGTTGA ATGAACTTCA GCATTCAAAT TTATGAATGA     1758
CAGTAGTCAC ACATAGTGCT GTTTATATAG TTTAGGAGTA AGAGTCTTGT     1808
TTTTTATTCA GATTGGGAAA TCCATTCCAT TTTGTGAATT GGGACATAGT     1858
TACAGCAGTG GAATAAGTAT TCATTTAGAA ATGTGAATGA GCAGTAAAAC     1908
TGATGACATA AAGAAATTAA AAGATATTTA ATTCTTGCTT ATACTCAGTC     1958
TATTCGGTAA AATTTTTTTT AAAAAATGTG CATACCTGGA TTTCCTTGGC     2008
TTCTTTGAGA ATGTAAGACA AATTAAATCT GAATAAATCA TTCTCCCTGT     2058
TCACTGGCTC ATTTATTCTC TATGCACTGA GCATTTGCTC TGTGGAAGGC     2108
CCTGGGTTAA TAGTGGAGAT GCTAAGGTAA GCCAGACTCA CCCCTACCCA     2158
CAGGGTAGTA AAGTCTAGGA GCAGCAGTCA TATAATTAAG GTGGAGAGAT     2208
GCCCTCTAAG ATGTAGAG                                       2226
```

(2) INFORMATION FOR SEQUENCE ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2305 base pairs
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:
- (A) NAME/KEY: MAGE-51 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

```
GGATCCAGGC CTTGCCAGGA GAAAGGTGAG GGCCCTGTGT GAGCACAGAG      50
GGGACCATTC ACCCCAAGAG GGTGGAGACC TCACAGATTC CAGCCTACCC     100
TCCTGTTAGC ACTGGGGGCC TGAGGCTGTG CTTGCAGTCT GCACCCTGAG     150
GGCCCATGCA TTCCTCTTCC AGGAGCTCCA GGAAACAGAC ACTGAGGCCT     200
TGGTCTGAGG CCGTGCCCTC AGGTCACAGA GCAGAGGAGA TGCAGACGTC     250
TAGTGCCAGC AGTGAACGTT TGCCTTGAAT GCACACTAAT GGCCCCCATC     300
GCCCCAGAAC ATATGGGACT CCAGAGCACC TGGCCTCACC CTCTCTACTG     350
TCAGTCCTGC AGAATCAGCC TCTGCTTGCT TGTGTACCCT GAGGTGCCCT     400
CTCACTTTTT CCTTCAGGTT CTCAGGGGAC AGGCTGACCA GGATCACCAG     450
GAAGCTCCAG AGGATCCCCA GGAGGCCCTA GAGGAGCACC AAAGGAGAAG     500
ATCTGTAAGT AAGCCTTTGT TAGAGCCTCC AAGGTTCAGT TTTTAGCTGA     550
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GGCTTCTCAC ATGCTCCCTC TCTCTCCAGG CCAGTGGGTC TCCATTGCCC        600
AGCTCCTGCC CACACTCCTG CCTGTTGCGG TGACCAGAGT CGTC              644
ATG TCT CTT GAG CAG AAG AGT CAG CAC TGC AAG CCT GAG GAA        686
GGC CTT GAC ACC CAA GAA GAG CCC TGG GCC TGG TGG GTG TGC        728
AGG CTG CCA CTA CTG AGG AGC AGG AGG CTG TGT CCT CCT CCT        770
CTC CTC TGG TCC CAG GCA CCC TGG GGG AGG TGC CTG CTG CTG        812
GGT CAC CAG GTC CTC TCA AGA GTC CTC AGG GAG CCT CCG CCA        854
TCC CCA CTG CCA TCG ATT TCA CTC TAT GGA GGC AAT CCA TTA        896
AGG GCT CCA GCA ACC AAG AAG AGG AGG GGC CAA GCA CCT CCC        938
CTG ACC CAG AGT CTG TGT TCC GAG CAG CAC TCA GTA AGA AGG        980
TGG CTG ACT TGA                                              992
TTCATTTTCT GCTCCTCAAG TATTAAGTCA AGGAGCCGGT CACAAAGGCA       1042
GAAATGCTGG AGAGCGTCAT CAAAAATTAC AAGCGCTGCT TTCCTGAGAT       1092
CTTCGGCAAA GCCTCCGAGT CCTTGCAGCT GGTCTTTGGC ATTGACGTGA       1142
AGGAAGCGGA CCCCACCAGC AACACCTACA CCCTTGTCAC CTGCCTGGGA       1192
CTCCTATGAT GGCCTGGTGG TTTAATCAGA TCATGCCCAA GACGGGCCTC       1242
CTGATAATCG TCTTGGGCAT GATTGCAATG GAGGGCAAAT GCGTCCCTGA       1292
GGAGAAAATC TGGGAGGAGC TGGGTGTGAT GAAGGTGTAT GTTGGGAGGG       1342
AGCACAGTGT CTGTGGGGAG CCCAGGAAGC TGCTCACCCA AGATTTGGTG       1392
CAGGAAAACT ACCTGGAGTA CCGCAGGTGC CCAGCAGTGA TCCCATATGC       1442
TATGAGTTAC TGTGGGGTCC AAGGGCACTC GCTGCTTGAA AGTACTGGAG       1492
CACGTGGTCA GGGTCAATGC AAGAGTTCTC ATTTCCTACC CATCCCTGCA       1542
TGAAGCAGCT TTGAGAGAGG AGGAAGAGGG AGTCTGAGCA TGAGCTGCAG       1592
CCAGGGCCAC TGCGAGGGGG GCTGGGCCAG TGCACCTTCC AGGGCTCCGT       1642
CCAGTAGTTT CCCCTGCCTT AATGTGACAT GAGGCCCATT CTTCTCTCTT       1692
TGAAGAGAGC AGTCAACATT CTTAGTAGTG GGTTTCTGTT CTATTGGATG       1742
ACTTTGAGAT TTGTCTTTGT TTCCTTTTGG AATTGTTCAA ATGTTCCTTT       1792
TAATGGGTGG TTGAATGAAC TTCAGCATTC AAATTTATGA ATGACAGTAG       1842
TCACACATAG TGCTGTTTAT ATAGTTTAGG AGTAAGAGTC TTGTTTTTTA       1892
TTCAGATTGG GAAATCCATT CCATTTTGTG AATTGGGACA TAGTTACAGC       1942
AGTGGAATAA GTATTCATTT AGAAATGTGA ATGAGCAGTA AAACTGATGA       1992
GATAAAGAAA TTAAAAGATA TTTAATTCTT GCCTTATACT CAGTCTATTC       2042
GGTAAAATTT TTTTTTAAAA ATGTGCATAC CTGGATTTCC TTGGCTTCTT       2092
TGAGAATGTA AGACAAATTA AATCTGAATA AATCATTCTC CCTGTTCACT       2142
GGCTCATTTA TTCTCTATGC ACTGAGCATT TGCTCTGTGG AAGGCCCTGG       2192
GTTAATAGTG GAGATGCTAA GGTAAGCCAG ACTCACCCCT ACCCACAGGG       2242
TAGTAAAGTC TAGGAGCAGC AGTCATATAA TTAAGGTGGA GAGATGCCCT       2292
CTAAGATGTA GAG                                              2305
```

(2) INFORMATION FOR SEQUENCE ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 225 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-6 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

```
TAT TTC TTT CCT GTG ATC TTC AGC AAA GCT TCC GAT TCC TTG        42
CAG CTG GTC TTT GGC ATC GAG CTG ATG GAA GTG GAC CCC ATC        84
GGC CAC GTG TAC ATC TTT GCC ACC TGC CTG GGC CTC TCC TAC       126
GAT GGC CTG CTG GGT GAC AAT CAG ATC ATG CCC AGG ACA GGC       168
TTC CTG ATA ATC ATC CTG GCC ATA ATC GCA AGA GAG GGC GAC       210
TGT GCC CCT GAG GAG                                          225
```

BNSDOCID: <WO 9523874A1>

(2) INFORMATION FOR SEQUENCE ID NO: 19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1947 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-7 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

TGAATGGACA ACAAGGGCCC CACACTCCCC AGAACACAAG GGACTCCAGA 50
GAGCCCAGCC TCACCTTCCC TACTGTCAGT CCTGCAGCCT CAGCCTCTGC 100
TGGCCGGCTG TACCCTGAGG TGCCCTCTCA CTTCCTCCTT CAGGTTCTCA 150
GCGGACAGGC CGGCCAGGAG GTCAGAAGCC CCAGGAGGCC CCAGAGGAGC 200
ACCGAAGGAG AAGATCTGTA AGTAGGCCTT TGTTAGGGCC TCCAGGGCGT 250
GGTTCACAAA TGAGGCCCCT CACAAGCTCC TTCTCTCCCC AGATCTGTGG 300
GTTCCTCCCC ATCGCCCAGC TGCTGCCCGC ACTCCAGCCT GCTGCCCTGA 350
CCAGAGTCAT CATGTCTTCT GAGCAGAGGA GTCAGCACTG CAAGCCTGAG 400
GATGCCTTGA GGCCCAAGGA CAGGAGGCTC TGGGCCTGGT GGGTGCGCAG 450
GCTCCCGCCA CCGAGGAGCA CGAGGCTGCC TCCTCCTTCA CTCTGATTGA 500
AGGCACCCTG GAGGAGGTGC CTGCTGCTGG GTCCCCCAGT CCTCCCCTGA 550
GTCTCAGGGT TCCTCCTTTT CCCTGACCAT CAGCAACAAC ACTCTATGGA 600
GCCAATCCAG TGAGGGCACC AGCAGCCGGG AAGAGGAGGG GCCAACCACC 650
TAGACACACC CCGCTCACCT GGCGTCCTTG TTCCA 685
ATG GGA AGG TGG CTG AGT TGG TTC GCT TCC TGC TGC ACA AGT 727
ATC GAG TCA AGG AGC TGG TCA CAA AGG CAG AAA TGC TGG ACA 769
GTG TCA TCA AAA ATT ACA AGC ACT AGT TTC CTT GTG ATC TAT 811
GGC AAA GCC TCA GAG TGC ATG CAG GTG ATG TTT GGC ATT GAC 853
ATG AAG GAA GTG GAC CCC GCG GCC ACT CCT ACG TCC TTG TCA 895
CCT GCT TGG GCC TCT CCT ACA ATG GCC TGC TGG GTG ATG ATC 937
AGA GCA TGC CCG AGA CCG GCC TTC TGA 964
TTATGGTCTT GACCATGATC TTAATGGAGG GCCACTGTGC CCCTGAGGAG 1014
GCAATCTGGG AAGCGTTGAG TGTAATGGTG TATGATGGGA TGGAGCAGTT 1064
TCTTTGGGCA GCTGAGGAAG CTGCTCACCC AAGATTGGGT GCAGGAAAAC 1114
TACCTGCAAT ACCGCCAGGT GCCCAGCAGT GATCCCCCGT GCTACCAGTT 1164
CCTGTGGGGT CCAAGGGCCC TCATTGAAAC CAGCTATGTG AAAGTCCTGG 1214
AGTATGCAGC CAGGGTCAGT ACTAAAGAGA GCATTTCCTA CCCATCCCTG 1264
CATGAAGAGG CTTTGGGAGA GGAGGAAGAG GGAGTCTGAG CAGAAGTTGC 1314
AGCCAGGGCC AGTGGGGCAG ATTGGGGGAG GGCCTGGGCA GTGCACGTTC 1364
CACACATCCA CCACCTTCCC TGTCCTGTTA CATGAGGCCC ATTCTTCACT 1414
CTGTGTTTGA AGAGAGCAGT CAATGTTCTC AGTAGCGGGG AGTGTGTTGG 1464
GTGTGAGGGA ATACAAGGTG GACCATCTCT CAGTTCCTGT TCTCTTGGGC 1514
GATTTGGAGG TTTATCTTTG TTTCCTTTTG CAGTCGTTCA AATGTTCCTT 1564
TTAATGGATG GTGTAATGAA CTTCAACATT CATTTCATGT ATGACAGTAG 1614
GCAGACTTAC TGTTTTTTAT ATAGTTAAAA GTAAGTGCAT TGTTTTTTAT 1664
TTATGTAAGA AAATCTATGT TATTTCTTGA ATTGGGACAA CATAACATAG 1714
CAGAGGATTA AGTACCTTTT ATAATGTGAA AGAACAAAGC GGTAAAATGG 1764
GTGAGATAAA GAAATAAAGA AATTAAATTG GCTGGGCACG GTGGCTCACG 1814
CCTGTAATCC CAGCACTTTA GGAGGCAGAG GCACGGGGAT CACGAGGTCA 1864
GGAGATCGAG ACCATTCTGG CTAACACAGT GAAACACCAT CTCTATTAAA 1914
AATACAAAAC TTAGCCGGGC GTGGTGGCGG GTG 1947

(2) INFORMATION FOR SEQUENCE ID NO: 20:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1810 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-8 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

GAGCTCCAGG AACCAGGCTG TGAGGTCTTG GTCTGAGGCA GTATCTTCAA 50
TCACAGAGCA TAAGAGGCCC AGGCAGTAGT AGCAGTCAAG CTGAGGTGGT 100

```
GTTTCCCCTG TATGTATACC AGAGGCCCCT CTGGCATCAG AACAGCAGGA      150
ACCCCACAGT TCCTGGCCCT ACCAGCCCTT TTGTCAGTCC TGGAGCCTTG      200
GCCTTTGCCA GGAGGCTGCA CCCTGAGATG CCCTCTCAAT TTCTCCTTCA      250
GGTTCGCAGA GAACAGGCCA GCCAGGAGGT CAGGAGGCCC CAGAGAAGCA      300
CTGAAGAAGA CCTGTAAGTA GACCTTTGTT AGGGCATCCA GGGTGTAGTA      350
CCCAGCTGAG GCCTCTCACA CGCTTCCTCT CTCCCCAGGC CTGTGGGTCT      400
CAATTGCCCA GCTCCGGCCC ACACTCTCCT GCTGCCCTGA CCTGAGTCAT      450
C                                                          451
ATG CTT CTT GGG CAG AAG AGT CAG CGC TAC AAG GCT GAG GAA     493
GGC CTT CAG GCC CAA GGA GAG GCA CCA GGG CTT ATG GAT GTG     535
CAG ATT CCC ACA GCT GAG GAG CAG AAG GCT GCA TCC TCC TCC     577
TCT ACT CTG ATC ATG GGA ACC CTT GAG GAG GTG ACT GAT TCT     619
GGG TCA CCA AGT CCT CCC CAG AGT CCT GAG GGT GCC TCC TCT     661
TCC CTG ACT GTC ACC GAC AGC ACT CTG TGG AGC CAA TCC GAT     703
GAG GGT TCC AGC AGC AAT GAA GAG GAG GGG CCA AGC ACC TCC     745
CCG GAC CCA GCT CAC CTG GAG TCC CTG TTC CGG GAA GCA CTT     787
GAT GAG AAA GTG GCT GAG TTA GTT CGT TTC CTG CTC CGC AAA     829
TAT CAA ATT AAG GAG CCG GTC ACA AAG GCA GAA ATG CTT GAG     871
AGT GTC ATC AAA AAT TAC AAG AAC CAC TTT CCT GAT ATC TTC     913
AGC AAA GCC TCT GAG TGC ATG CAG GTG ATC TTT GGC ATT GAT     955
GTG AAG GAA GTG GAC CCT GCC GGC CAC TCC TAC ATC CTT GTC     997
ACC TGC CTG GGC CTC TCC TAT GAT GGC CTG CTG GGT GAT GAT    1039
CAG AGT ACG CCC AAG ACC GGC CTC CTG ATA ATC GTC CTG GGC    1081
ATG ATC TTA ATG GAG GGC AGC CGC GCC CCG GAG GAG GCA ATC    1123
TGG GAA GCA TTG AGT GTG ATG GGG GCT GTA TGA                1156
TGGGAGGGAG CACAGTGTCT ATTGGAAGCT CAGGAAGCTG CTCACCCAAG     1206
AGTGGGTGCA GGAGAACTAC CTGGAGTACC GCCAGGCGCC CGGCAGTGAT     1256
CCTGTGCGCT ACGAGTTCCT GTGGGGTCCA AGGGCCCTTG CTGAAACCAG     1306
CTATGTGAAA GTCCTGGAGC ATGTGGTCAG GGTCAATGCA AGAGTTCGCA     1356
TTTCCTACCC ATCCCTGCAT GAAGAGGCTT TGGGAGAGGA GAAAGGAGTT     1406
TGAGCAGGAG TTGCAGCTAG GGCCAGTGGG GCAGGTTGTG GGAGGGCCTG     1456
GGCCAGTGCA CGTTCCAGGG CCACATCCAC CACTTTCCCT GCTCTGTTAC     1506
ATGAGGCCCA TTCTTCACTC TGTGTTTGAA GAGAGCAGTC ACAGTTCTCA     1556
GTAGTGGGGA GCATGTTGGG TGTGAGGGAA CACAGTGTGG ACCATCTCTC     1606
AGTTCCTGTT CTATTGGGCG ATTTGGAGGT TTATCTTTGT TTCCTTTTGG     1656
AATTGTTCCA ATGTTCCTTC TAATGGATGG TGTAATGAAC TTCAACATTC     1706
ATTTTATGTA TGACAGTAGA CAGACTTACT GCTTTTTATA TAGTTTAGGA     1756
GTAAGAGTCT TGCTTTTCAT TTATACTGGG AAACCCATGT TATTTCTTGA     1806
ATTC                                                      1810
```

(2) INFORMATION FOR SEQUENCE ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1412 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-9 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

```
TCTGAGACAG TGTCCTCAGG TCGCAGAGCA GAGGAGACCC AGGCAGTGTC      50
AGCAGTGAAG GTGAAGTGTT CACCCTGAAT GTGCACCAAG GGCCCCACCT     100
GCCCCAGCAC ACATGGGACC CCATAGCACC TGGCCCCATT CCCCCTACTG     150
TCACTCATAG AGCCTTGATC TCTGCAGGCT AGCTGCACGC TGAGTAGCCC     200
TCTCACTTCC TCCCTCAGGT TCTCGGGACA GGCTAACCAG GAGGACAGGA     250
GCCCCAAGAG GCCCCAGAGC AGCACTGACG AAGACCTGTA AGTCAGCCTT     300
TGTTAGAACC TCCAAGGTTC GGTTCTCAGC TGAAGTCTCT CACACACTCC     350
CTCTCTCCCC AGGCCTGTGG GTCTCCATCG CCCAGCTCCT GCCCACGCTC     400
CTGACTGCTG CCCTGACCAG AGTCATC                              427
ATG TCT CTC GAG CAG AGG AGT CCG CAC TGC AAG CCT GAT GAA     469
GAC CTT GAA GCC CAA GGA GAG GAC TTG GGC CTG ATG GGT GCA     511
CAG GAA CCC ACA GGC GAG GAG GAG GAG ACT ACC TCC TCC TCT     553
GAC AGC AAG GAG GAG GAG GTG TCT GCT GCT GGG TCA TCA AGT     595
```

```
CCT CCC CAG AGT CCT CAG GGA GGC GCT TCC TCC TCC ATT TCC      637
GTC TAC TAC ACT TTA TGG AGC CAA TTC GAT GAG GGC TCC AGC      679
AGT CAA GAA GAG GAA GAG CCA AGC TCC TCG GTC GAC CCA GCT      721
CAG CTG GAG TTC ATG TTC CAA GAA GCA CTG AAA TTG AAG GTG      763
GCT GAG TTG GTT CAT TTC CTG CTC CAC AAA TAT CGA GTC AAG      805
GAG CCG GTC ACA AAG GCA GAA ATG CTG GAG AGC GTC ATC AAA      847
AAT TAC AAG CGC TAC TTT CCT GTG ATC TTC GGC AAA GCC TCC      889
GAG TTC ATG CAG GTG ATC TTT GGC ACT GAT GTG AAG GAG GTG      931
GAC CCC GCC GGC CAC TCC TAC ATC CTT GTC ACT GCT CTT GGC      973
CTC TCG TGC GAT AGC ATG CTG GGT GAT GGT CAT AGC ATG CCC     1015
AAG GCC GCC CTC CTG ATC ATT GTC CTG GGT GTG ATC CTA ACC     1057
AAA GAC AAC TGC GCC CCT GAA GAG GTT ATC TGG GAA GCG TTG     1099
AGT GTG ATG GGG GTG TAT GTT GGG AAG GAG CAC ATG TTC TAC     1141
GGG GAG CCC AGG AAG CTG CTC ACC CAA GAT TGG GTG CAG GAA     1183
AAC TAC CTG GAG TAC CGG CAG GTG CCC GGC AGT GAT CCT GCG     1225
CAC TAC GAG TTC CTG TGG GGT TCC AAG GCC CAC GCT GAA ACC     1267
AGC TAT GAG AAG GTC ATA AAT TAT TTG GTC ATG CTC AAT GCA     1309
AGA GAG CCC ATC TGC TAC CCA TCC CTT TAT GAA GAG GTT TTG     1351
GGA GAG GAG CAA GAG GGA GTC TGA                            1375
GCACCAGCCG CAGCCGGGGC CAAAGTTTGT GGGGTCA                   1412
```

(2) INFORMATION FOR SEQUENCE ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 920 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:

(A) NAME/KEY: MAGE-10 gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

```
ACCTGCTCCA GGACAAAGTG GACCCCACTG CATCAGCTCC ACCTACCCTA       50
CTGTCAGTCC TGGAGCCTTG GCCTCTGCCG GCTGCATCCT GAGGAGCCAT      100
CTCTCACTTC CTTCTTCAGG TTCTCAGGGG ACAGGGAGAG CAAGAGGTCA      150
AGAGCTGTGG GACACCACAG AGCAGCACTG AAGGAGAAGA CCTGTAAGTT      200
GGCCTTTGTT AGAACCTCCA GGGTGTGGTT CTCAGCTGTG GCCACTTACA      250
CCCTCCCTCT CTCCCCAGGC CTGTGGGTCC CCATCGCCCA AGTCCTGCCC      300
ACACTCCCAC CTGCTACCCT GATCAGAGTC ATC                       333
ATG CCT CGA GCT CCA AAG CGT CAG CGC TGC ATG CCT GAA GAA      375
GAT CTT CAA TCC CAA AGT GAG ACA CAG GGC CTC GAG GGT GCA      417
CAG GCT CCC CTG GCT GTG GAG GAG GAT GCT TCA TCA TCC ACT      459
TCC ACC AGC TCC TCT TTT CCA TCC TCT TTT CCC TCC TCC TCC      501
TCT TCC TCC TCC TCC TCC TGC TAT CCT CTA ATA CCA AGC ACC      543
CCA GAG GAG GTT TCT GCT GAT GAT GAG ACA CCA AAT CCT CCC      585
CAG AGT GCT CAG ATA GCC TGC TCC TCC CCC TCG GTC GTT GCT      627
TCC CTT CCA TTA GAT CAA TCT GAT GAG GGC TCC AGC AGC CAA      669
AAG GAG GAG AGT CCA AGC ACC CTA CAG GTC CTG CCA GAC AGT      711
GAG TCT TTA CCC AGA AGT GAG ATA GAT GAA AAG GTG ACT GAT      753
TTG GTG CAG TTT CTG CTC TTC AAG TAT CAA ATG AAG GAG CCG      795
ATC ACA AAG GCA GAA ATA CTG GAG AGT GTC ATA AAA AAT TAT      837
GAA GAC CAC TTC CCT TTG TTG TTT AGT GAA GCC TCC GAG TGC      879
ATG CTG CTG GTC TTT GGC ATT GAT GTA AAG GAA GTG GAT CC       920
```

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(2) INFORMATION FOR SEQUENCE ID NO: 23:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1107 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: MAGE-11 gene
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

```
AGAGAACAGG CCAACCTGGA GGACAGGAGT CCCAGGAGAA CCCAGAGGAT        50
CACTGGAGGA GAACAAGTGT AAGTAGGCCT TTGTTAGATT CTCCATGGTT       100
CATATCTCAT CTGAGTCTGT TCTCACGCTC CCTCTCTCCC CAGGCTGTGG       150
GGCCCCATCA CCCAGATATT TCCCACAGTT CGGCCTGCTG ACCTAACCAG       200
AGTCATCATG CCTCTTGAGC AAAGAAGTCA GCACTGCAAG CCTGAGGAAG       250
CCTTCAGGCC CAAGAAGAAG ACCTGGGCCT GGTGGGTGCA CAGGCTCTCC       300
AAGCTGAGGA GCAGGAGGCT GCCTTCTTCT CCTCTACTCT GAATGTGGGC       350
ACTCTAGAGG AGTTGCCTGC TGCTGAGTCA CCAAGTCCTC CCCAGAGTCC       400
TCAGGAAGAG TCCTTCTCTC CCACTGCCAT GGATGCCATC TTTGGGAGCC       450
TATCTGATGA GGGCTCTGGC AGCCAAGAAA AGGAGGGGCC AAGTACCTCG       500
CCTGACCTGA TAGACCCTGA GTCCTTTTCC CAAGATATAC TACATGACAA       550
GATAATTGAT TTGGTTCATT TATTCTCCGC AAGTATCGAG TCAAGGGGCT       600
GATCACAAAG GCAGAA                                           616
ATG CTG GGG AGT GTC ATC AAA AAT TAT GAG GAC TAC TTT CCT      658
GAG ATA TTT AGG GAA GCC TCT GTA TGC ATG CAA CTG CTC TTT      700
GGC ATT GAT GTG AAG GAA GTG GAC CCC ACT AGC CAC TCC TAT      742
GTC CTT GTC ACC TCC CTC AAC CTC TCT TAT GAT GGC ATA CAG      784
TGT AAT GAG CAG AGC ATG CCC AAG TCT GGC CTC CTG ATA ATA      826
GTC CTG GGT GTA ATC TTC ATG GAG GGG AAC TGC ATC CCT GAA      868
GAG GTT ATG TGG GAA GTC CTG AGC ATT ATG GGG GTG TAT GCT      910
GGA AGG GAG CAC TTC CTC TTT GGG GAG CCC AAG AGG CTC CTT      952
ACC CAA AAT TGG GTG CAG GAA AAG TAC CTG GTG TAC CGG CAG      994
GTG CCC GGC ACT GAT CCT GCA TGC TAT GAG TTC CTG TGG GGT     1036
CCA AGG GCC CAC GCT GAG ACC AGC AAG ATG AAA GTT CTT GAG     1078
TAC ATA GCC AAT GCC AAT GGG AGG GAT CC                      1107
```

(2) INFORMATION FOR SEQUENCE ID NO: 24:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2150 base pairs
(B) TYPE: nucleic acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: genomic DNA
(ix) FEATURE:
(A) NAME/KEY: smage-I
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

```
TCTGTCTGCA TATGCCTCCA CTTGTGTGTA GCAGTCTCAA ATGGATCTCT        50
CTCTACAGAC CTCTGTCTGT GTCTGGCACC CTAAGTGGCT TTGCATGGGC       100
ACAGGTTTCT GCCCCTGCAT GGAGCTTAAA TAGATCTTTC TCCACAGGCC       150
TATACCCCTG CATTGTAAGT TTAAGTGGCT TTATGTGGAT ACAGGTCTCT       200
GCCCTTGTAT GCAGGCCTAA GTTTTTCTGT CTGCTTAACC CCTCCAAGTG       250
AAGCTAGTGA AAGATCTAAC CCACTTTTGG AAGTCTGAAA CTAGACTTTT       300
ATGCAGTGGC CTAACAAGTT TTAATTTCTT CCACAGGGTT TGCAGAAAAG       350
AGCTTGATCC ACGAGTTCAG AAGTCCTGGT ATGTTCCTAG AAAG             394
ATG TTC TCC TGG AAA GCT TCA AAA GCC AGG TCT CCA TTA AGT      436
CCA AGG TAT TCT CTA CCT GGT AGT ACA GAG GTA CTT ACA GGT      478
TGT CAT TCT TAT CCT TCC AGA TTC CTG TCT GCC AGC TCT TTT      520
ACT TCA GCC CTG AGC ACA GTC AAC ATG CCT AGG GGT CAA AAG      565
AGT AAG ACC CGC TCC CGT GCA AAA CGA CAG CAG TCA CGC AGG      604
GAG GTT CCA GTA GTT CAG CCC ACT GCA GAG GAA GCA GGG TCT      646
TCT CCT GTT GAC CAG AGT GCT GGG TCC AGC TTC CCT GGT GGT      688
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```
TCT GCT CCT CAG GGT GTG AAA ACC CCT GGA TCT TTT GGT GCA      730
GGT GTA TCC TGC ACA GGC TCT GGT ATA GGT GGT AGA AAT GCT      772
GCT GTC CTG CCT GAT ACA AAA AGT TCA GAT GGC ACC CAG GCA      814
GGG ACT TCC ATT CAG CAC ACA CTG AAA GAT CCT ATC ATG AGG      856
AAG GCT AGT GTG CTG ATA GAA TTC CTG CTA GAT AAA TTT AAG      898
ATG AAA GAA GCA GTT ACA AGG AGT GAA ATG CTG GCA GTA GTT      940
AAC AAG AAG TAT AAG GAG CAA TTC CCT GAG ATC CTC AGG AGA      982
ACT TCT GCA CGC CTA GAA TTA GTC TTT GGT CTT GAG TTG AAG     1024
GAA ATT GAT CCC AGC ACT CAT TCC TAT TTG CTG GTA GGC AAA     1066
CTG GGT CTT TCC ACT GAG GGA AGT TTG AGT AGT AAC TGG GGG     1108
TTG CCT AGG ACA GGT CTC CTA ATG TCT GTC CTA GGT GTG ATC     1150
TTC ATG AAG GGT AAC CGT GCC ACT GAG CAA GAG GTC TGG CAA     1192
TTT CTG CAT GGA GTG GGG GTA TAT GCT GGG AAG AAG CAC TTG     1234
ATC TTT GGC GAG CCT GAG GAG TTT ATA AGA GAT GTA GTG CGG     1276
GAA AAT TAC CTG GAG TAC CGC CAG GTA CCT GGC AGT GAT CCC     1314
CCA AGC TAT GAG TTC CTG TGG GGA CCC AGA GCC CAT GCT GAA     1360
ACA ACC AAG ATG AAA GTC CTG GAA GTT TTA GCT AAA GTC AAT     1402
GGC ACA GTC CCT AGT GCC TTC CCT AAT CTC TAC CAG TTG GCT     1444
CTT AGA GAT CAG GCA GGA GGG GTG CCA AGA AGG AGA GTT CAA     1486
GGC AAG GGT GTT CAT TCC AAG GCC CCA TCC CAA AAG TCC TCT     1528
AAC ATG TAG                                               1537
TTGAGTCTGT TCTGTTGTGT TTGAAAAACA GTCAGGCTCC TAATCAGTAG     1587
AGAGTTCATA GCCTACCAGA ACCAACATGC ATCCATTCTT GGCCTGTTAT     1637
ACATTAGTAG AATGGAGGCT ATTTTTGTTA CTTTTCAAAT GTTTGTTTAA     1687
CTAAACAGTG CTTTTTGCCA TGCTTCTTGT TAACTGCATA AAGAGGTAAC     1737
TGTCACTTGT CAGATTAGGA CTTGTTTTGT TATTTGCAAC AAACTGGAAA     1787
ACATTATTTT GTTTTTACTA AAACATTGTG TAACATTGCA TTGGAGAAGG     1837
GATTGTCATG GCAATGTGAT ATCATACAGT GGTGAAACAA CAGTGAAGTG     1887
GGAAAGTTTA TATTGTTAAT TTTGAAAATT TTATGAGTGT GATTGCTGTA     1937
TACTTTTTTC TTTTTTGTAT AATGCTAAGT GAAATAAAGT TGGATTTGAT     1987
GACTTTACTC AAATTCATTA GAAAGTAAAT CGTAAAACTC TATTACTTTA     2037
TTATTTTCTT CAATTATGAA TTAAGCATTG GTTATCTGGA AGTTTCTCCA     2087
GTAGCACAGG ATCTAGTATG AAATGTATCT AGTATAGGCA CTGACAGTGA     2137
GTTATCAGAG TCT                                            2150
```

(2) INFORMATION FOR SEQUENCE ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2099 base pairs
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: genomic DNA

(ix) FEATURE:
- (A) NAME/KEY: smage-II

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

```
ACCTTATTGG GTCTGTCTGC ATATGCCTCC ACTTGTGTGT AGCAGTCTCA       50
AATGGATCTC TCTCTACAGA CCTCTGTCTG TGTCTGGCAC CCTAAGTGGC      100
TTTGCATGGG CACAGGTTTC TGCCCCTGCA TGGAGCTTAA ATAGATCTTT      150
CTCCACAGGC CTATACCCCT GCATTGTAAG TTTAAGTGGC TTTATGTGGA      200
TACAGGTCTC TGCCCTTGTA TGCAGGCCTA AGTTTTTCTG TCTGCTTAGC      250
CCCTCCAAGT GAAGCTAGTG AAAGATCTAA CCCACTTTTG GAAGTCTGAA      300
ACTAGACTTT TATGCAGTGG CCTAACAAGT TTTAATTTCT TCCACAGGGT      350
TTGCAGAAAA GAGCTTGATC CACGAGTTCG GAAGTCCTGG TATGTTCCTA      400
GAAAGATGTT CTCCTGGAAA GCTTCAAAAG CCAGGTCTCC ATTAAGTCCA      450
AGGTATTCTC TACCTGGTAG TACAGAGGTA CTTACAGGTT GTCATTCTTA      500
TCTTTCCAGA TTCCTGTCTG CCAGCTCTTT TACTTCAGCC CTGAGCACAG      550
TCAACATGCC TAGGGGTCAA AAGAGTAAGA CCCGCTCCCG TGCAAAACGA      600
CAGCAGTCAC GCAGGGAGGT TCCAGTAGTT CAGCCCACTG CAGAGGAAGC      650
AGGGTCTTCT CCTGTTGACC AGAGTGCTGG GTCCAGCTTC CCTGGTGGTT      700
CTGCTCCTCA GGGTGTGAAA ACCCCTGGAT CTTTTGGTGC AGGTGTATCC      750
TGCACAGGCT CTGGTATAGG TGGTAGAAAT GCTGCTGTCC TGCCTGATAC      800
AAAAAGTTCA GATGGCACCC AGGCAGGGAC TTCCATTCAG CACACACTGA      850
AAGATCCTAT CATGAGGAAG GCTAGTGTGC TGATAGAATT CCTGCTAGAT      900
```

```
AAGTTTAAGA TGAAAGAAGC AGTTACAAGG AGTGAAATGC TGGCAGTAGT      950
TAACAAGAAG TATAAGGAGC AATTCCCTGA GATCCTCAGG AGAACTTCTG     1000
CACGCCTAGA ATTAGTCTTT GGTCTTGAGT TGAAGGAAAT TGATCCCAGC     1050
ACTCATTCCT ATTTGCTGGT AGGCAAACTG GGTCTTTCCA CTGAGGGAAG     1100
TTTGAGTAGT AACTGGGGGT TGCCTAGGAC AGGTCTCCTA ATGTCTGTCC     1150
TAGGTGTGAT CTTCATGAAG GGTAACCGTG CCACTGAGCA AGAGGTCTGG     1200
CAATTTCTGC ATGGAGTGGG GGTATATGCT GGGAAGAAGC ACTTGATCTT     1250
TGGCGAGCCT GAGGAGTTTA TAAGAGATGT AGTGCGGGAA AATTACCTGG     1300
AGTACCGCCA GGTACCTGGC AGTGATCCCC CAAGCTATGA GTTCCTGTGG     1350
GGACCCAGAG CCCATGCTGA AACAACCAAG ATGAAAGTCC TGGAAGTTTT     1400
AGCTAAAGTC AATGGCACAG TCCCTAGTGC CTTCCCTAAT CTCTACCAGT     1450
TGGCTCTTAG AGATCAGGCA GGAGGGGTGC CAAGAAGGAG AGTTCAAGGC     1500
AAGGGTGTTC ATTCCAAGGC CCCATCCCAA AAGTCCTCTA ACATGTAGTT     1550
GAGTCTGTTC TGTTGTGTTT GAAAAACAGT CAGGCTCCTA ATCAGTAGAG     1600
AGTTCATAGC CTACCAGAAC CAACATGCAT CCATTCTTGG CCTGTTATAC     1650
ATTAGTAGAA TGGAGGCTAT TTTTGTTACT TTTCAAATGT TTGTTTAACT     1700
AAACAGTGCT TTTTGCCATG CTTCTTGTTA ACTGCATAAA GAGGTAACTG     1750
TCACTTGTCA GATTAGGACT TGTTTTGTTA TTTGCAACAA ACTGGAAAAC     1800
ATTATTTTGT TTTTACTAAA ACATTGTGTA ACATTGCATT GGAGAAGGGA     1850
TTGTCATGGC AATGTGATAT CATACAGTGG TGAAACAACA GTGAAGTGGG     1900
AAAGTTTATA TTGTTAGTTT TGAAAATTTT ATGAGTGTGA TTGCTGTATA     1950
CTTTTTTCTT TTTTGTATAA TGCTAAGTGA AATAAAGTTG GATTTGATGA     2000
CTTTACTCAA ATTCATTAGA AAGTAAATCA TAAAACTCTA TTACTTTATT     2050
ATTTTCTTCA ATTATTAATT AAGCATTGGT TATCTGGAAG TTTCTCCAG      2099
```

(2) INFORMATION FOR SEQUENCE ID NO: 26:
- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 9 amino acids
  - (B) TYPE: amino acids
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

```
Glu Ala Asp Pro Thr Gly His Ser Tyr
 1               5
```

(2) INFORMATION FOR SEQUENCE ID NO: 27:
- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

```
TGGAGGACCA GAGGCCCCC                                        19
```

(2) INFORMATION FOR SEQUENCE ID NO: 28:
- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

```
GGACGATTAT CAGGAGGCCT GC                                    22
```

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(2) INFORMATION FOR SEQUENCE ID NO: 29:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

GAGCAGACAG GCCAACCG 18

(2) INFORMATION FOR SEQUENCE ID NO: 30:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

AAGGACTCTG CGTCAGGC 18

(2) INFORMATION FOR SEQUENCE ID NO: 31:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

CTAGAGGAGC ACCAAAGGAG AAG 23

(2) INFORMATION FOR SEQUENCE ID NO: 32:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

TGCTCGGAAC ACAGACTCTG G 21

(2) INFORMATION FOR SEQUENCE ID NO: 33:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

TGGAGGACCA GAGGCCCCC 19

(2) INFORMATION FOR SEQUENCE ID NO: 34:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

CAGGATGATT ATCAGGAAGC CTGT 24

(2) INFORMATION FOR SEQUENCE ID NO: 35:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

CAGAGGAGCA CCGAAGGAGA A 21

(2) INFORMATION FOR SEQUENCE ID NO: 36:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

CAGGTGAGCG GGGTGTGTC 19

(2) INFORMATION FOR SEQUENCE ID NO: 37:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

CCCCAGAGAA GCACTGAAGA AG 22

(2) INFORMATION FOR SEQUENCE ID NO: 38:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

GGTGAGCTGG GTCCGGG 17

(2) INFORMATION FOR SEQUENCE ID NO: 39:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

CCCCAGAGCA GCACTGACG 19

(2) INFORMATION FOR SEQUENCE ID NO: 40:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

CAGCTGAGCT GGGTCGACC 19

(2) INFORMATION FOR SEQUENCE ID NO: 41:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

CACAGAGCAG CACTGAAGGA G 21

(2) INFORMATION FOR SEQUENCE ID NO: 42:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

CTGGGTAAAG ACTCACTGTC TGG 23

(2) INFORMATION FOR SEQUENCE ID NO: 43:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

GAGAACCCAG AGGATCACTG GA 22

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(2) INFORMATION FOR SEQUENCE ID NO: 44:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

GGGAAAAGGA CTCAGGGTCT ATC 23

(2) INFORMATION FOR SEQUENCE ID NO: 45:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

GGTGGAAGTG GTCCGCATCG 20

(2) INFORMATION FOR SEQUENCE ID NO: 46:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

GCCCTCCACT GATCTTTAGC AA 22

(2) INFORMATION FOR SEQ ID NO: 47:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

CGGCCGAAGG AACCTGACCC AG 22

(2) INFORMATION FOR SEQ ID NO: 48:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

GCTGGAACCC TCACTGGGTT GCC 23

BNSDOCID: <WO 9523874A1>

(2) INFORMATION FOR SEQ ID NO: 49:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

AAGTAGGACC CGAGGCACTG 20

(2) INFORMATION FOR SEQ ID NO: 50:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

GAAGAGGAAG AAGCGGTCTG 20

(2) INFORMATION FOR SEQ ID NO: 51:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

TGGAGGACCA GAGGCCCCC 19

(2) INFORMATION FOR SEQ ID NO: 52:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

GGACGATTAT CAGGAGGCCT GC 22

(2) INFORMATION FOR SEQ ID NO: 53:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

ACTCAGCTCC TCCCAGATTT 20

(2) INFORMATION FOR SEQ ID NO: 54:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

GAAGAGGAGG GGCCAAG 17

(2) INFORMATION FOR SEQ ID NO: 55:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

TCTTGTATCC TGGAGTCC 18

(2) INFORMATION FOR SEQ ID NO: 56:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

TTGCCAAGAT CTCAGGAA 18

BNSDOCID: <WO 9523874A1>

Claims:

1. Isolated nucleic acid molecule useful as a primer in specifically determining expression of a member of the MAGE family of tumor rejection antigen precursor.

2. The isolated nucleic acid molecule of claim 1, selected from the group consisting of one of SEQ ID NOS: 27-48.

3. A kit useful in determining expression of a MAGE tumor rejection antigen precursor, comprising at least one part of SEQ ID NOS: 27 and 28, 29 and 30, 31 and 32, 33 and 34, 35 and 36, 37 and 38, 39 and 40, 41 and 42, 43 and 44, 45 and 46, and 47-48.

4. Method for determining expression of a MAGE tumor rejection antigen precursor in a cell comprising contacting said cell sample with at least one of the nucleic acid molecules of claim 2 and determining hybridization of said nucleic acid molecule to a target as a determination of expression of MAGE tumor rejection antigen precursor.

5. Method of claim 3, wherein said expression of said tumor rejection antigen precursor is a determination of presence, regression of spread of cancer.

6. The method of claim 5, wherein said cancer is melanoma.

7. The method of claim 5, wherein said cancer is lung adenocarcinoma, said method comprising contacting said sample with a pair of: SEQ ID NOS: 27 and 28, SEQ ID NOS: 47 and 48, or SEQ ID NOS: 49 and 50.

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8. The method of claim 4, wherein said cancer is a head squamous cell carcinoma, a neck squamous cell carcinoma, a prostate carcinoma, and a bladder tumor, the method comprising contacting sample with SEQ ID NOS: 27 AND 28, SEQ ID NOS: 29 and 30, SEQ ID NOS: 47 and 48, or SEQ ID NOS: 49 and 50.

9. The method of claim 4, wherein said cancer is a bladder tumor, the method comprising contacting said sample with SEQ ID NOS: 47 and 48, SEQ ID NOS: 49 and 50, or SEQ ID NOS: 51 and 52, followed by amplification.

10. The method of claim 4, wherein said MAGE tumor rejection antigen precursor is MAGE-1, MAGE-2, MAGE-3 or MAGE-4.

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AMENDED CLAIMS

[received by the International Bureau on 27 June 1995 (27.06.95); original claims 1-10 replaced by amended claims 1,9 (2 pages)]

1. Isolated nucleic acid molecule useful as a primer in specifically determining expression of a member of the MAGE group of tumor rejection antigen precursors, selected from the group consisting of SEQ ID NOS: 27-46.

2. Kit useful in determining expression of a MAGE tumor rejection antigen precursor, comprising at least one pair of:

SEQ ID NOS: 27 and 28
SEQ ID NOS: 29 and 30
SEQ ID NOS: 31 and 32
SEQ ID NOS: 33 and 34
SEQ ID NOS: 35 and 36
SEQ ID NOS: 37 and 38
SEQ ID NOS: 39 and 40
SEQ ID NOS: 41 and 42
SEQ ID NOS: 43 and 44
SEQ ID NOS: 45 and 46.

3. Method for determining expression of a MAGE tumor rejection antigen precursor in a cell comprising contacting said cell sample with at least one isolated nucleic acid molecule of claim 1 and determining hybridization of said nucleic acid molecule to a target as a determination of expression of MAGE tumor rejection antigen precursor.

4. The method of claim 3, wherein said expression of said tumor rejection antigen precursor is a determination of presence, regression of spread of cancer.

5. The method of claim 4, wherein said cancer is melanoma.

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6. The method of claim 4, comprising contacting said sample with SEQ ID NOS: 27 and 28.

7. The method of claim 4, wherein said cancer is a bead squamous cell carcinoma, a neck squamous cell carcinoma, a prostate carcinoma and a bladder tumor, the method comprising contacting said sample with SEQ ID NOS: 27 and 28 or SEQ ID NOS: 29 and 30.

8. The method of claim 4, wherein said cancer is a bladder tumor, said method comprising contacting said sample with SEQ ID NOS: 51 and 52, followed by amplification.

9. The method of claim 4, wherein said MAGE tumor rejection antigen precursor is MAGE-1, MAGE-2, MAGE-3 or MAGE-4.

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FIG. 1A

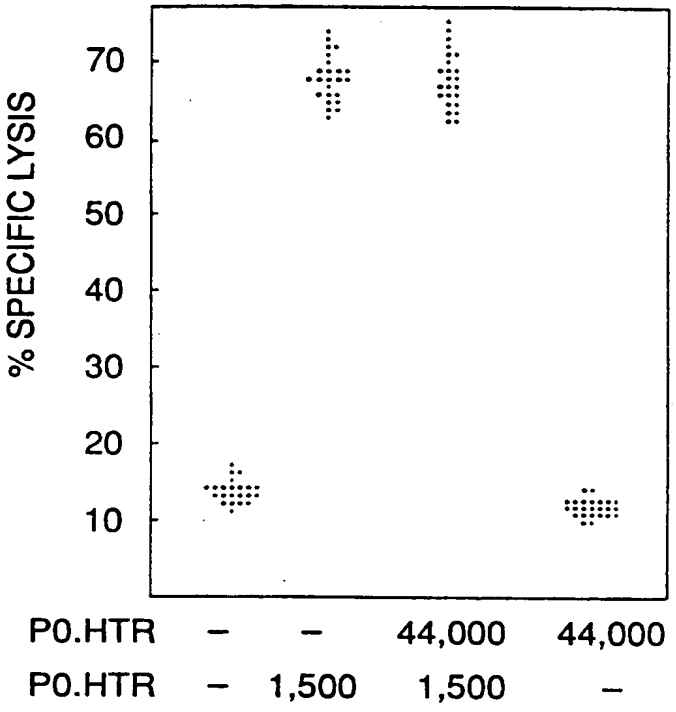

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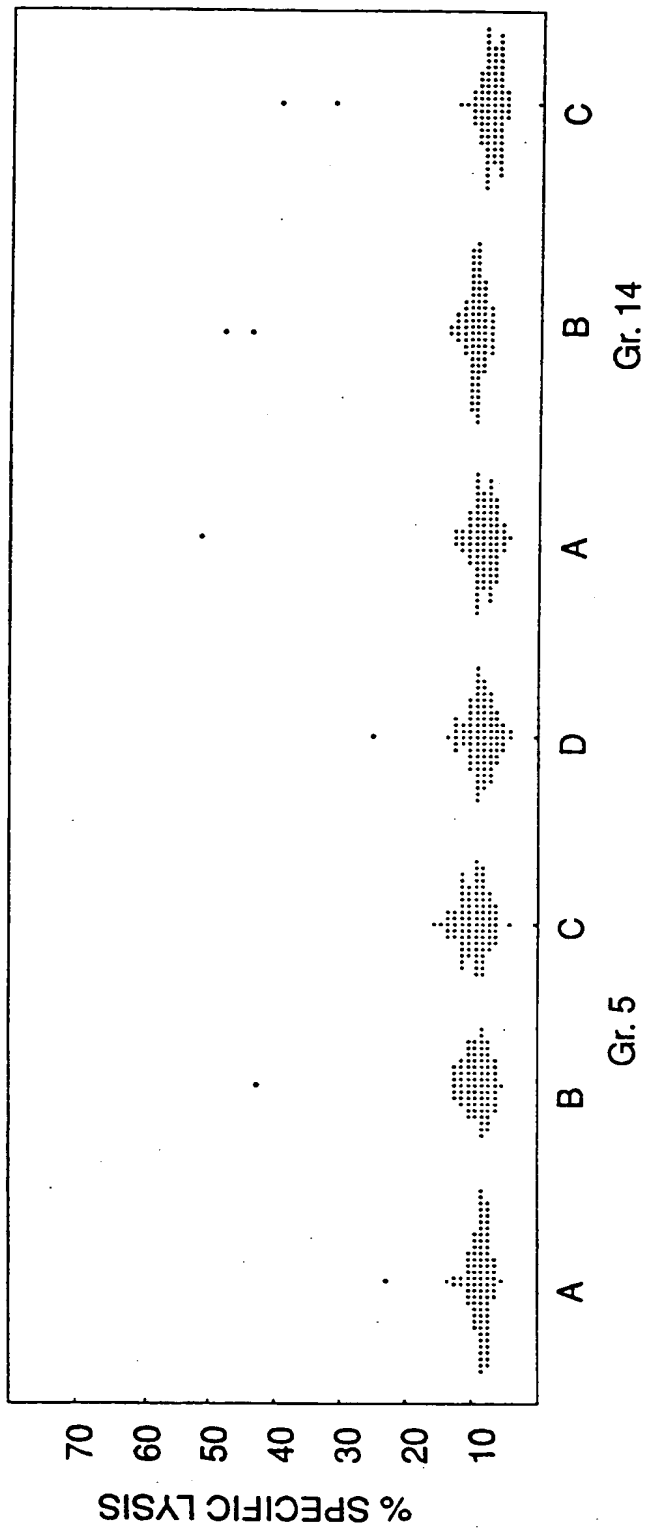
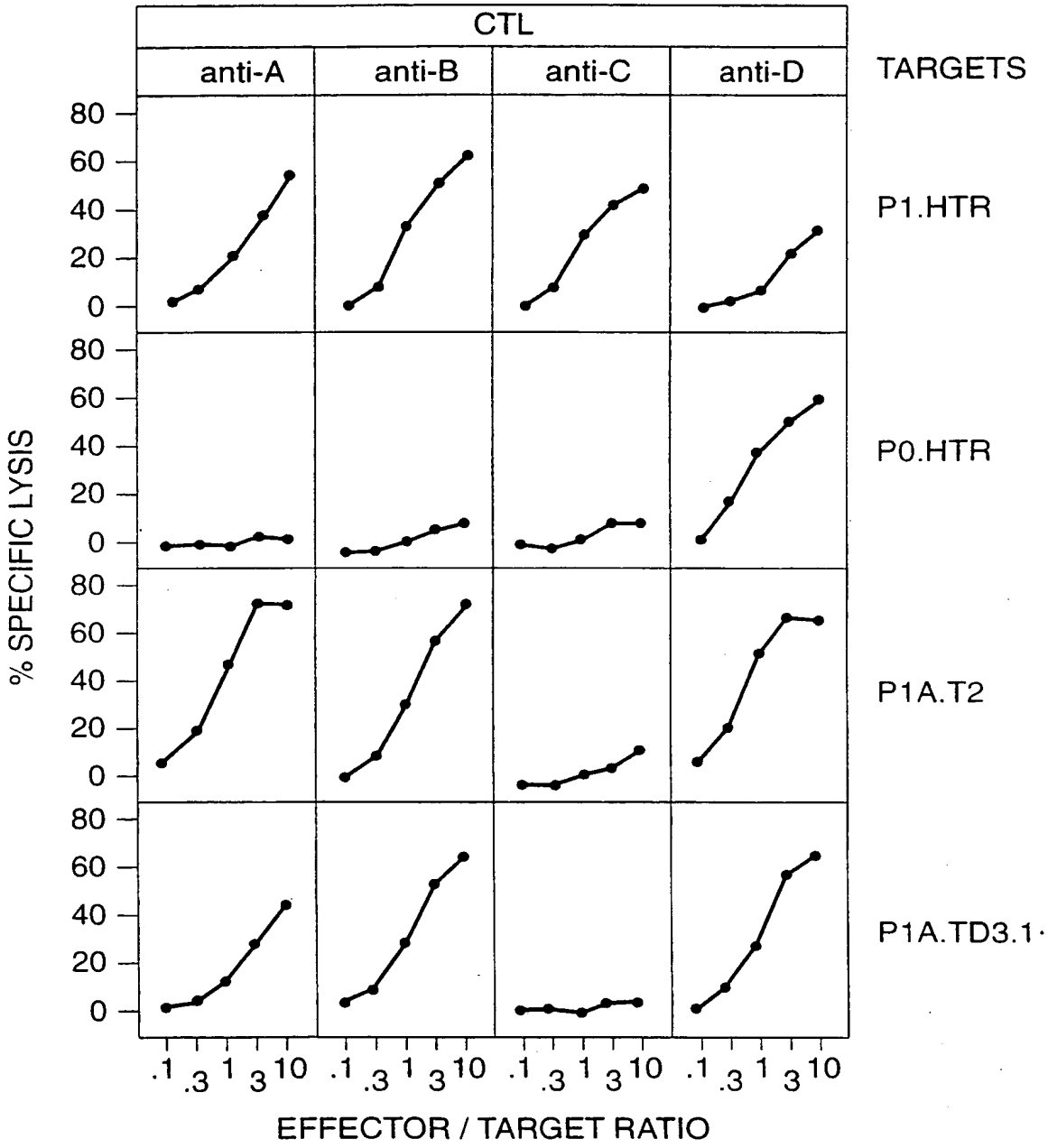
FIG. 1B



BNSDOCID: <WO 9523874A1>

FIG. 2



BNSDOCID: <WO 9523874A1>

FIG. 3

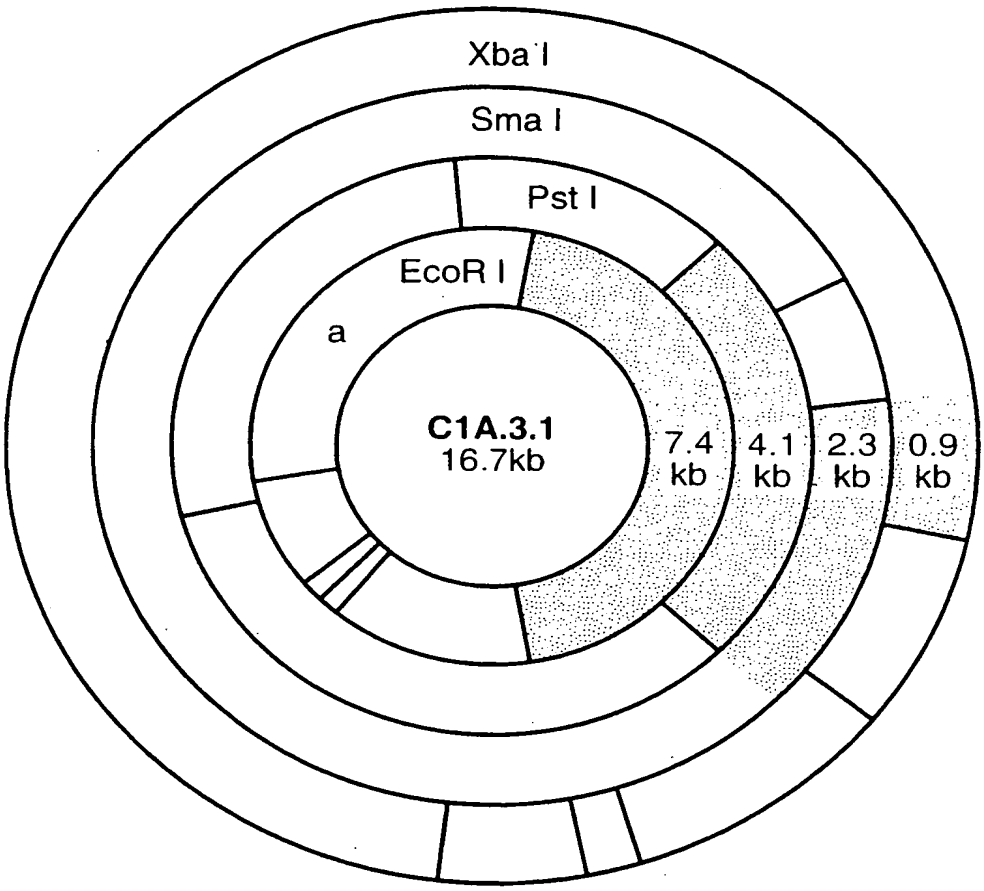


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FIG. 4

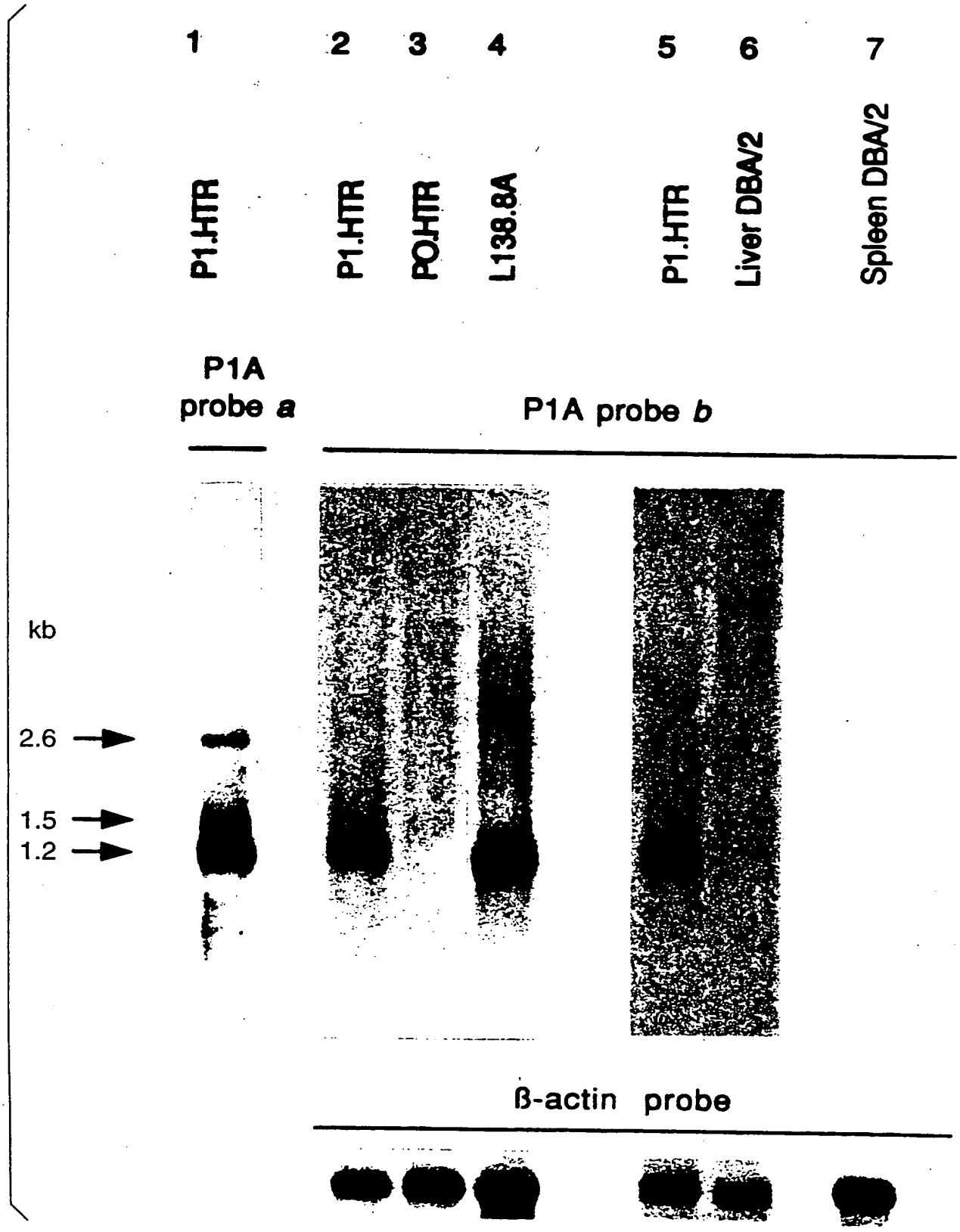

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FIG. 5

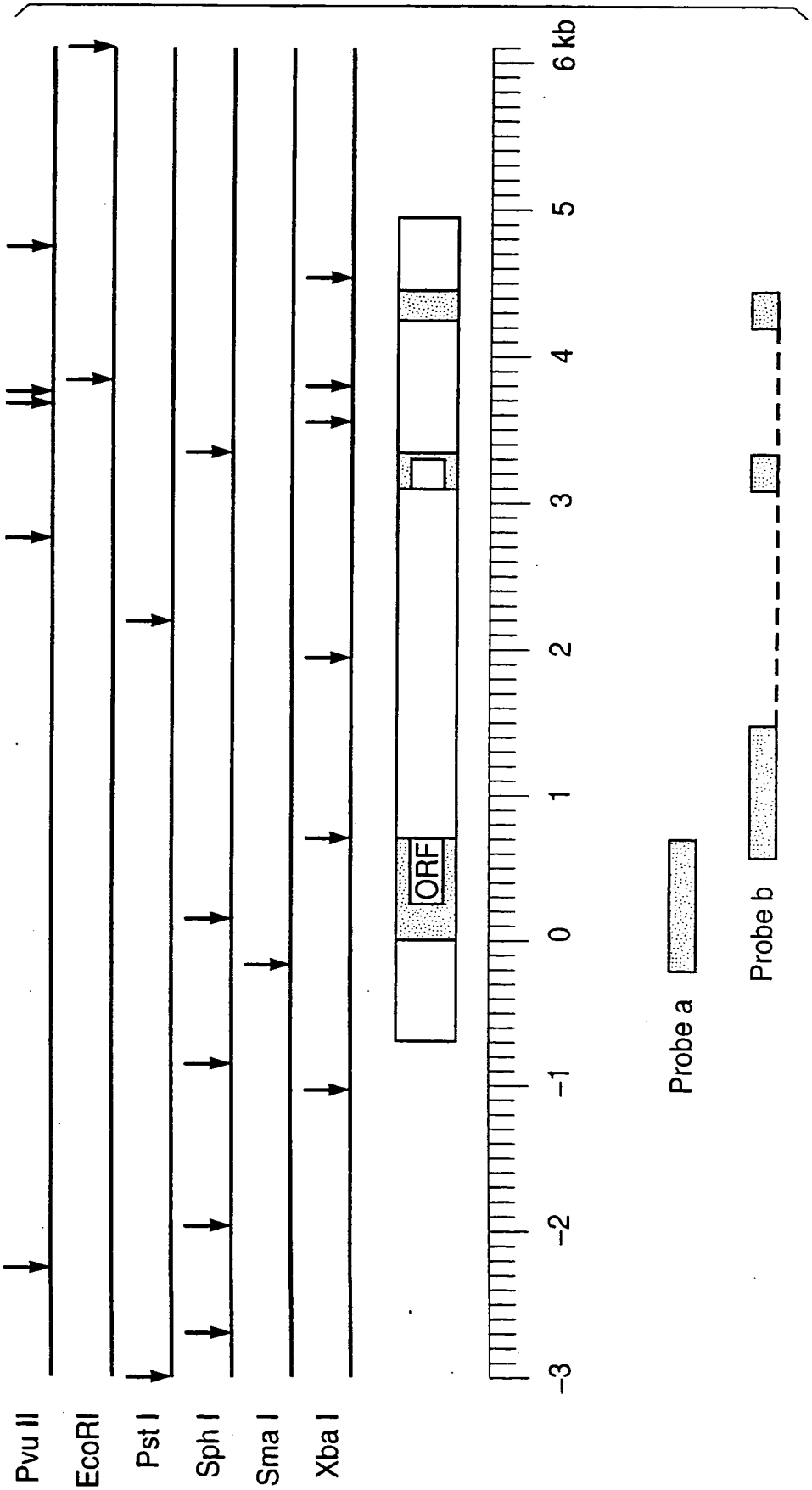

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FIG. 6

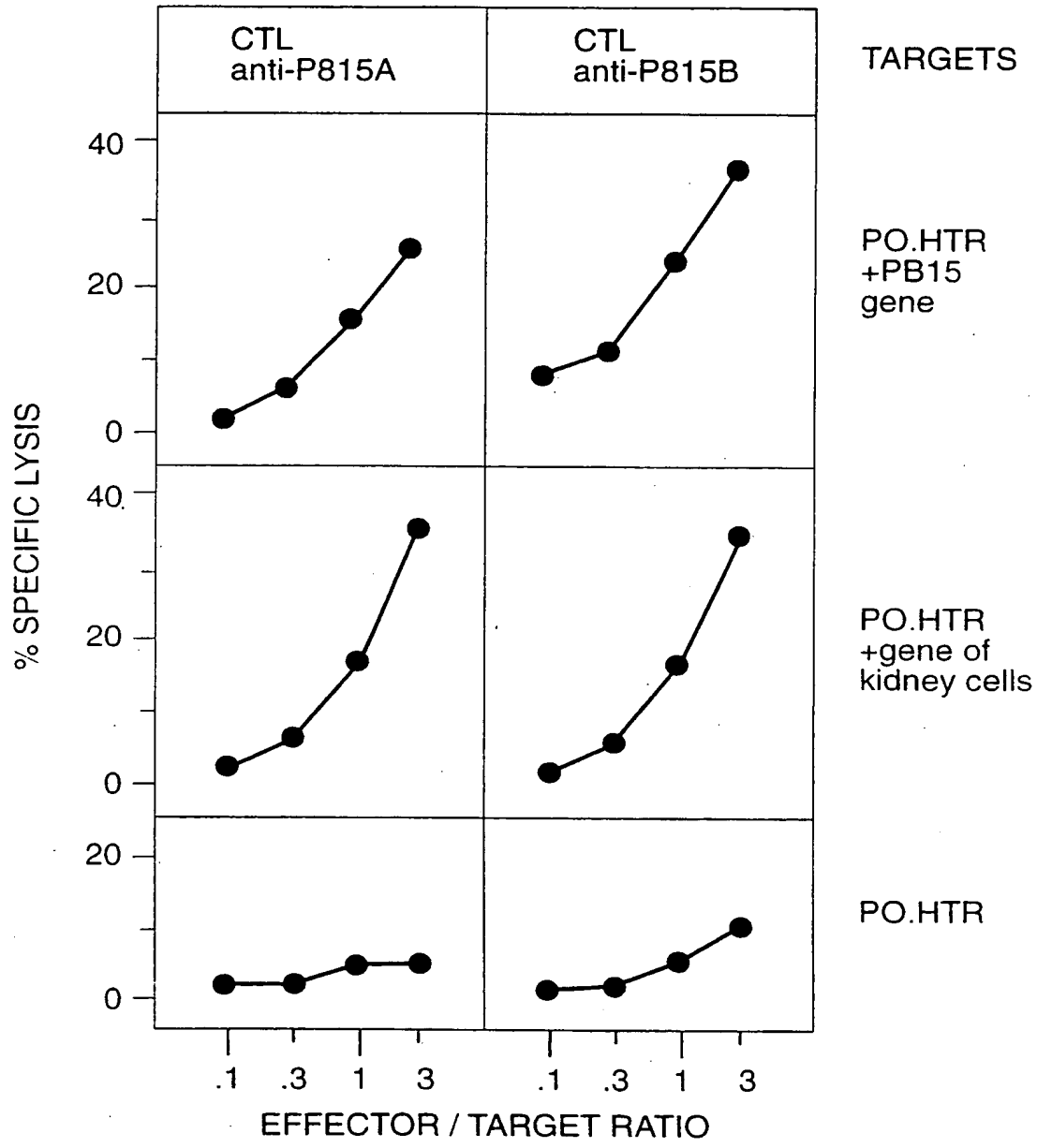

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FIG. 7

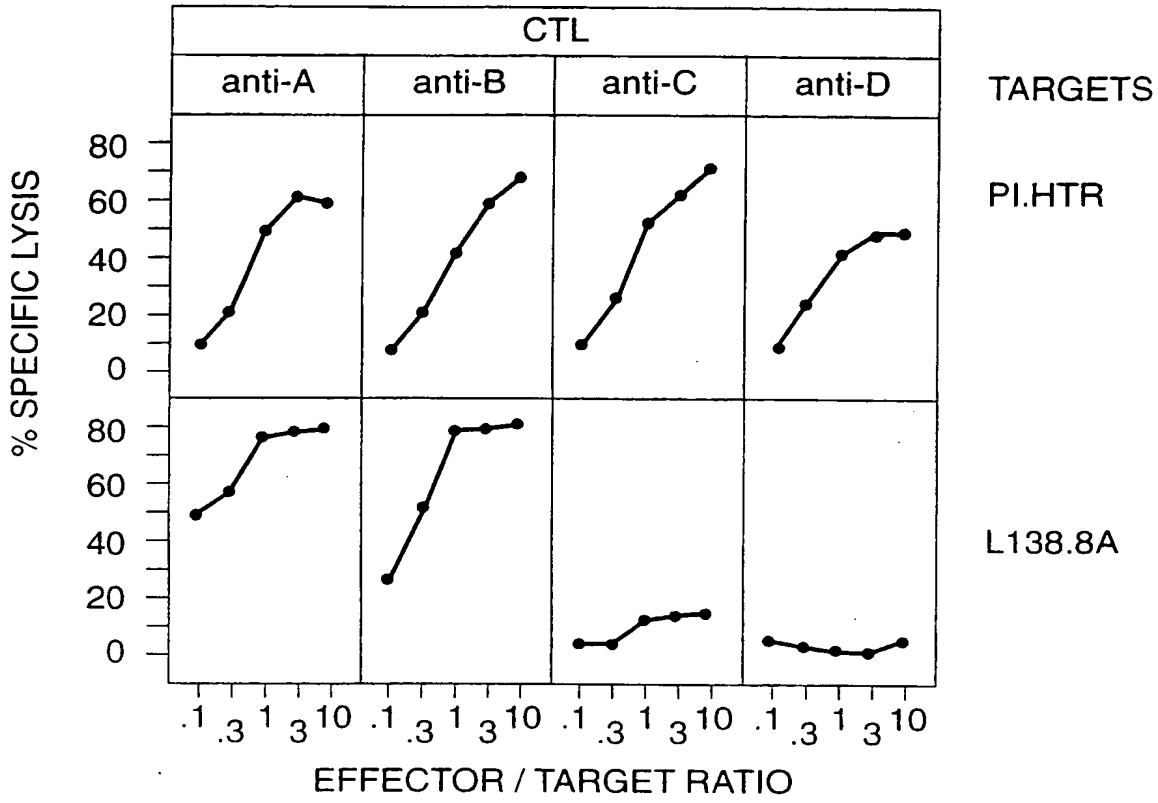
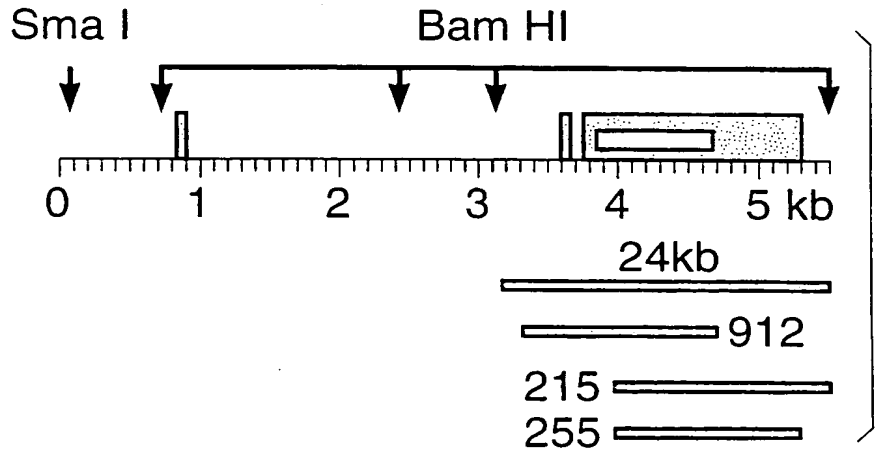


FIG. 8



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BNSDOCID: <WO 9523874A1>

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FIG. 9

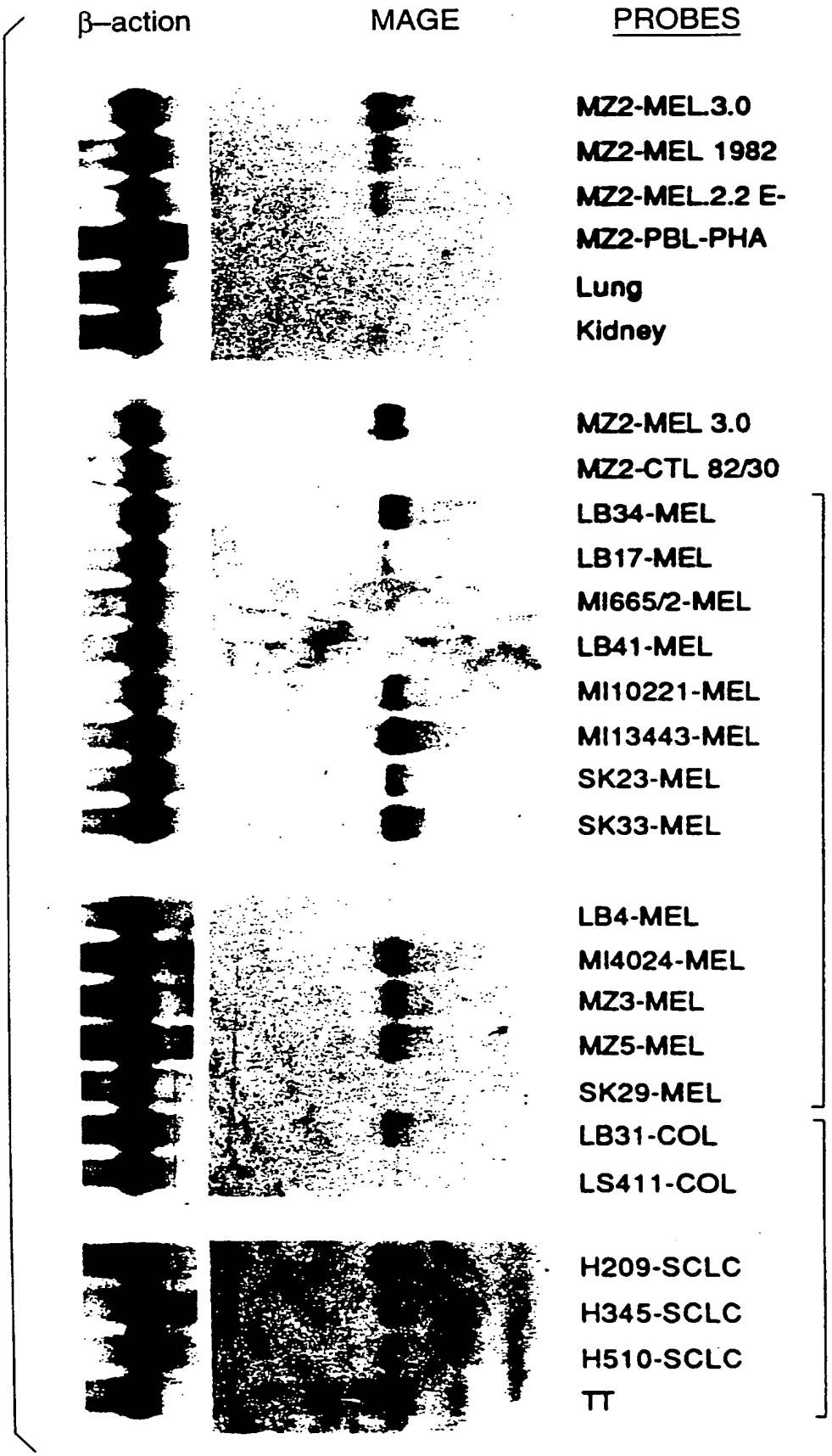

FIG. 10

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FIG. 11A

		EXPRSSION OF MAGE GENE FAMILY				RECOGNITION BY ANI-E CTL		
		Northern blot probed with cross-reactive MAGE-1 probe*	cDNA-PCR product probed with oligonucleotide specific for:			tested by:		Expression of antigen MZ2-E after transfection**
			MAGE-1	MAGE-2	MAGE-3†	TNF release‡	Lysis§	
Cells of patient MZ2	melanoma cell line MZ2-MEL.3.0	+	++++	++++	++++	+	+	
	tumor sample MZ2 (1982)	+	+++	+++	+++			
	antigen-loss variant MZ2-MEL.2.2	+	−	+++	+++	−	−	
	CTL clone MZ2-CTL.82/30	−	−	−	−			
	PHA-activated blood lymphocytes	−	−	−	−			
Normal tissues	Liver	−	−	−	−			
	Muscle	−	−	−	−			
	Skin	−	−	−	−			
	Lung	−	−	−	−			
	Brain	−	−	−	−			
	Kidney	−	−	−	−			
Melanoma cell lines of HLA-A1 patients	LB34-MEL	+	++	++++	++++	+	+−	
	MI665/2-MEL	−	−	−	−	−	−	+
	MI10221-MEL	+	−	++	+++	−	−	+
	MI13443-MEL	+	+++	++++	++++	+	+	
	SK33-MEL	+	−	++++	++++	−	−	−
	SK23-MEL	+	−	++++	++++	−	−	+

* Data obtained in the conditions of figure 5.
† Data obtained as described in figure 6.
‡ TNF release by CTL 82/30 after stimulation with the tumor cells as described in (11).
§ Lysis of 51 Cr labelled target by CTL 82/30 in the conditions of figure 1.
** Cells transfected with the 2.4 kb fragment of gene MAGE-1 were tested for their ability fo stimulate TNF release by CTL 82/30

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FIG. 11B

		EXPRSSION OF MAGE GENE FAMILY				RECOGNITION BY ANI-E CTL		
		Northern blot probed with cross-reactive MAGE-1 probe*	cDNA-PCR product probed with oligonucleotide specific for:			tested by:		Expression of antigen MZ2-E after transfection**
			MAGE-1	MAGE-2	MAGE-3†	TNF release‡	Lysis§	
Melanoma cell lines of other patients	LB17-MEL	+	+	++++	++++	−	−	−
	LB33-MEL	+	−	+++	+++	−	−	−
	LB4-MEL	−	−	−	−	−	−	
	LB41-MEL	−	−	−	−	−	−	
	MI4024-MEL	+	+++	++++	++++	−	−	
	SK29-MEL	−	−	−	−	−	−	
	MZ3-MEL	+	+	++++	++++	−	−	
	MZ5-MEL	+	−	++++	++++	−	−	
Melanoma tumor sample	BB5-MEL	+	+++	++	+++			
Other tumor cell lines	small cell lung cancer H209	+	−	++++	++++			
	small cell lung cancer H345	+	−	++++	++++			
	small cell lung cancer H510	+	−	++++	++++			
	small cell lung cancer LB11	+	+	++++	++++			
	bronchial squamous cell carcinoma LB37	+	−	−	+++			
	thyroid medullary carcinoma TT	+	++++	+++	++++			
	colon carcinoma LB31	+	−	+++	++++			
	colon carcinoma LS411	−	−	−	−	−		
Other tumor samples	chronic myeloid leukemia LLC5	−	−	−	−			
	acute myeloid leukemia TA	−	−	−	−			

* Data obtained in the conditions of figure 5.
† Data obtained as described in figure 6.
‡ TNF release by CTL 82/30 after stimulation with the tumor cells as described in (11).
§ Lysis of 51 Cr labelled target by CTL 82/30 in the conditions of figure 1.
** Cells transfected with the 2.4 kb fragment of gene MAGE-1 were tested for their ability fo stimulate TNF release by CTL 82/30

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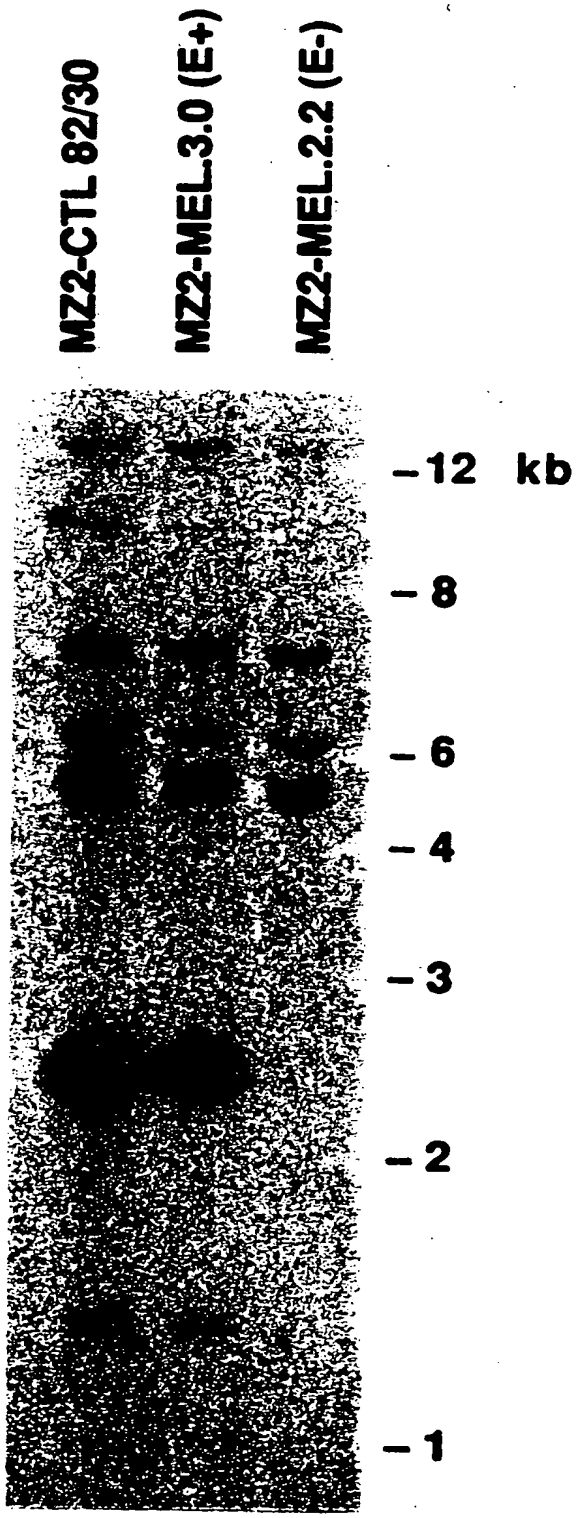
PCT/US95/02203

FIG. 12



BNSDOCID: <WO 9523874A1>

FIG. 13

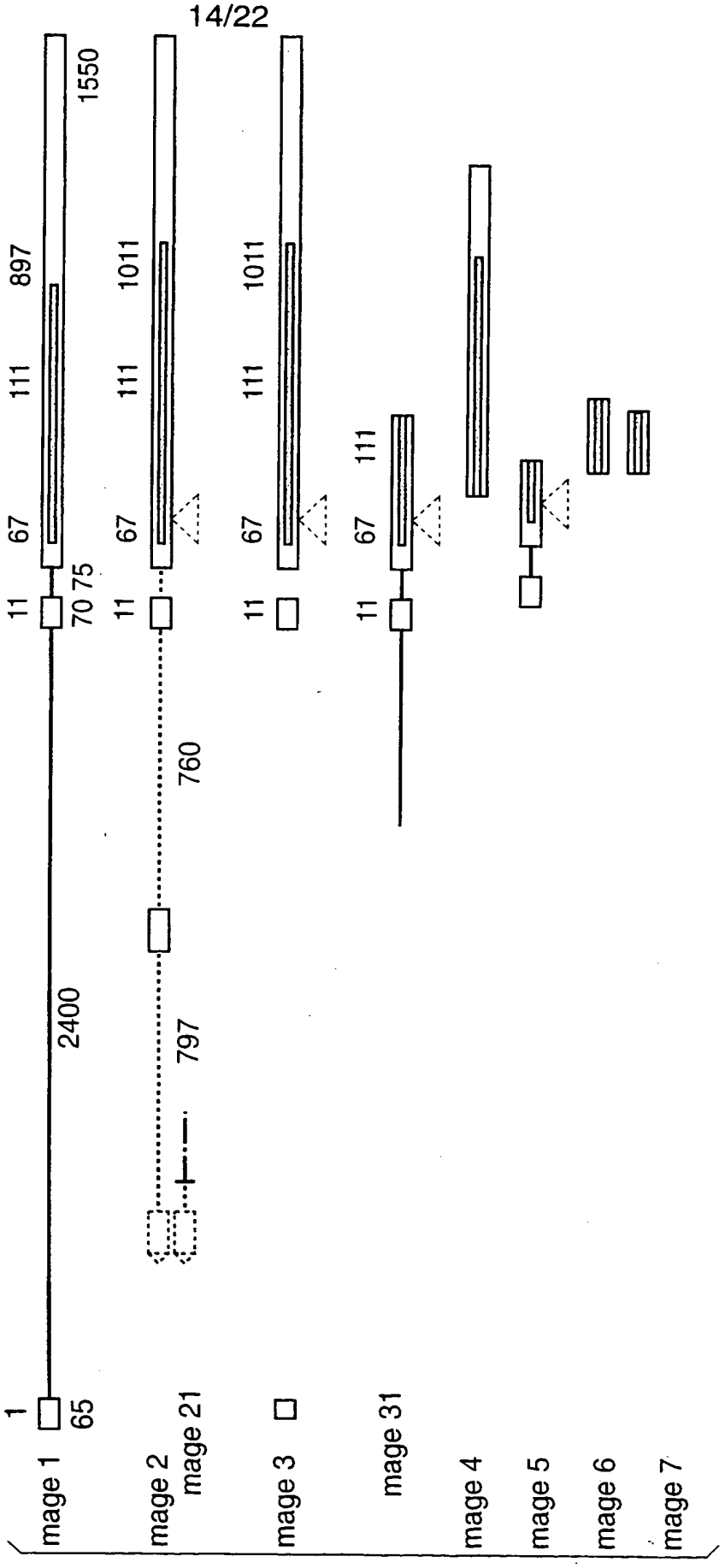


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FIG. 14B

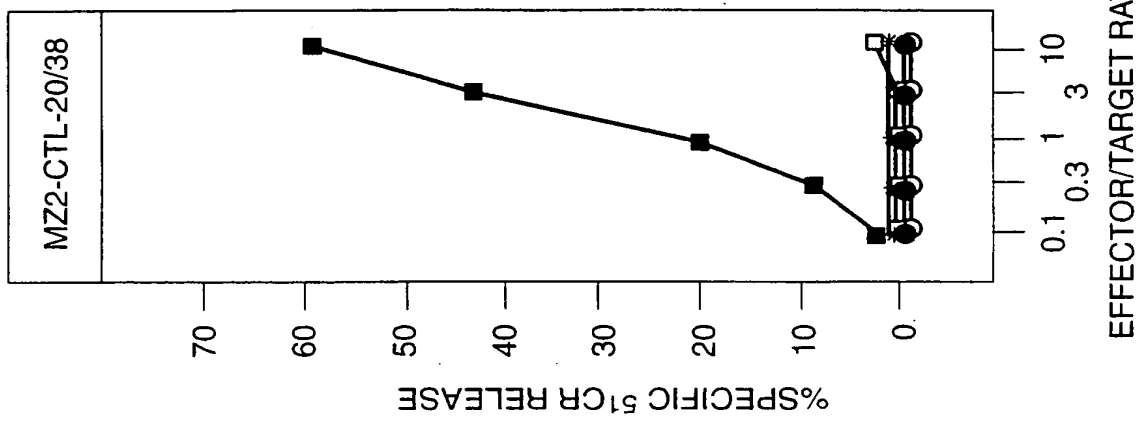
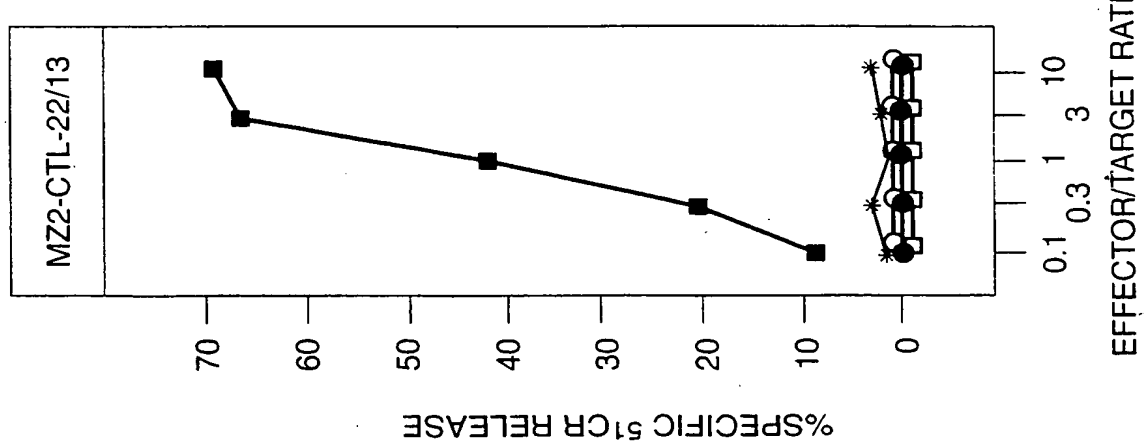


FIG. 14A



BNSDOCID: <WO 9523874A1>

FIG. 15A

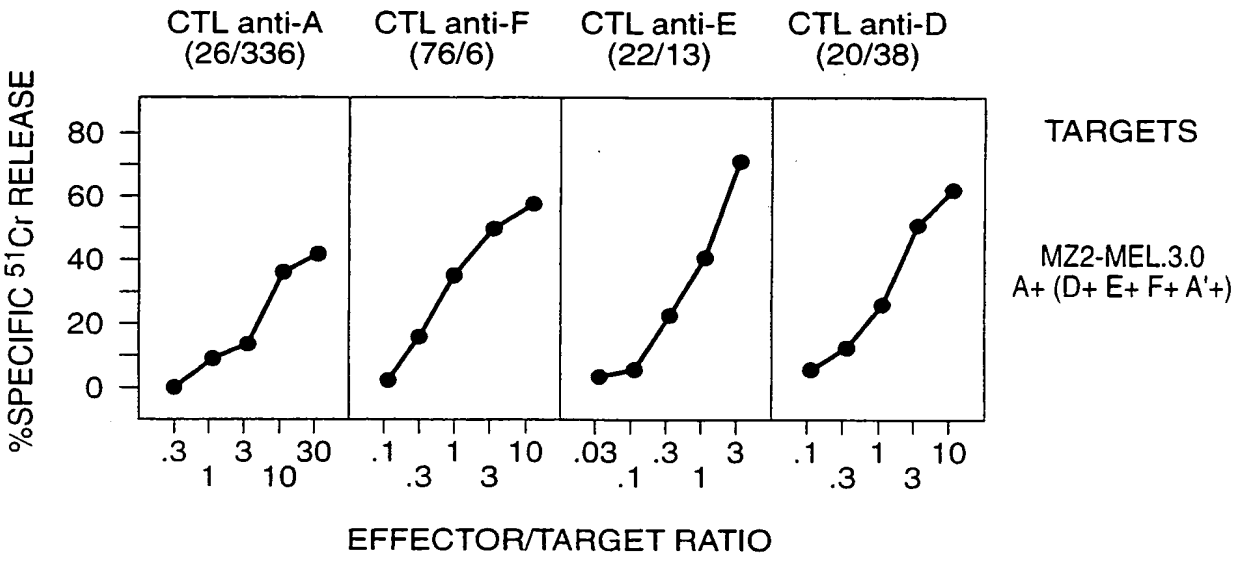
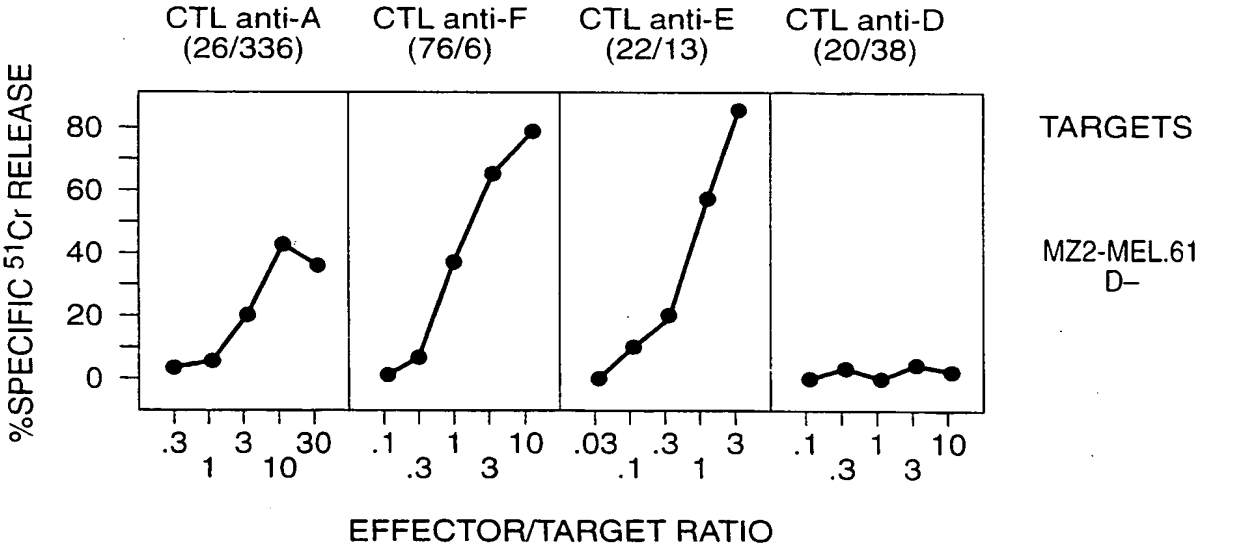


FIG. 15B



BNSDOCID: <WO 9523874A1>

FIG. 15C

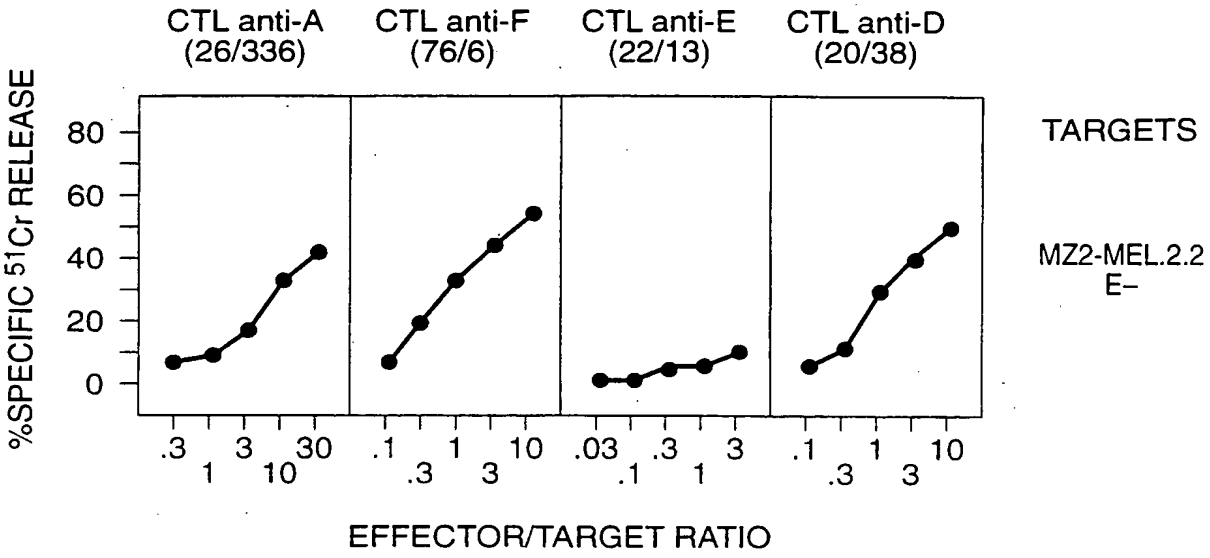
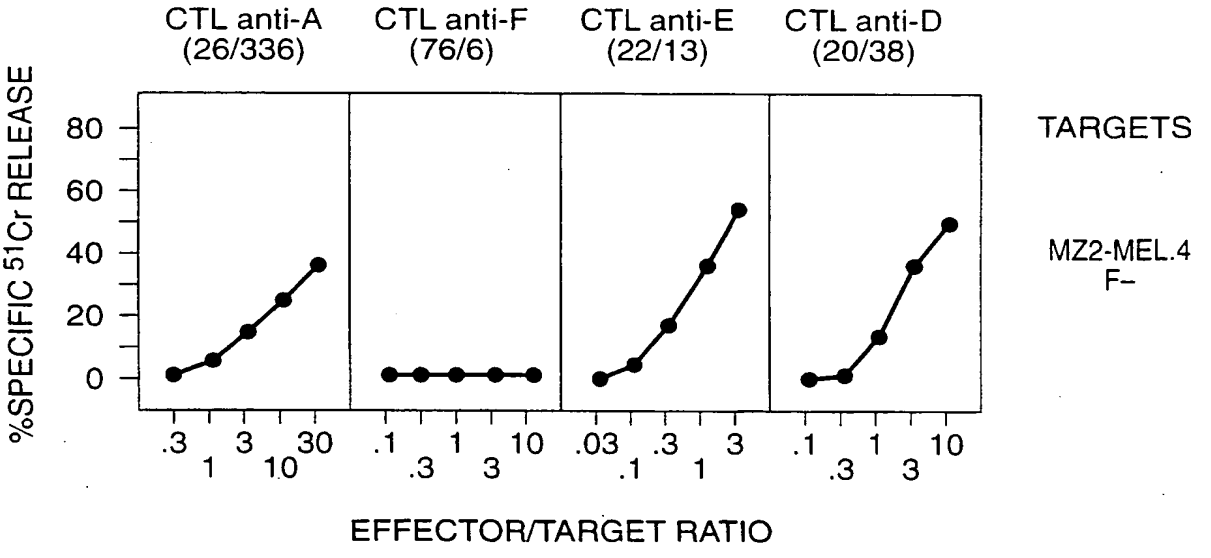


FIG. 15D



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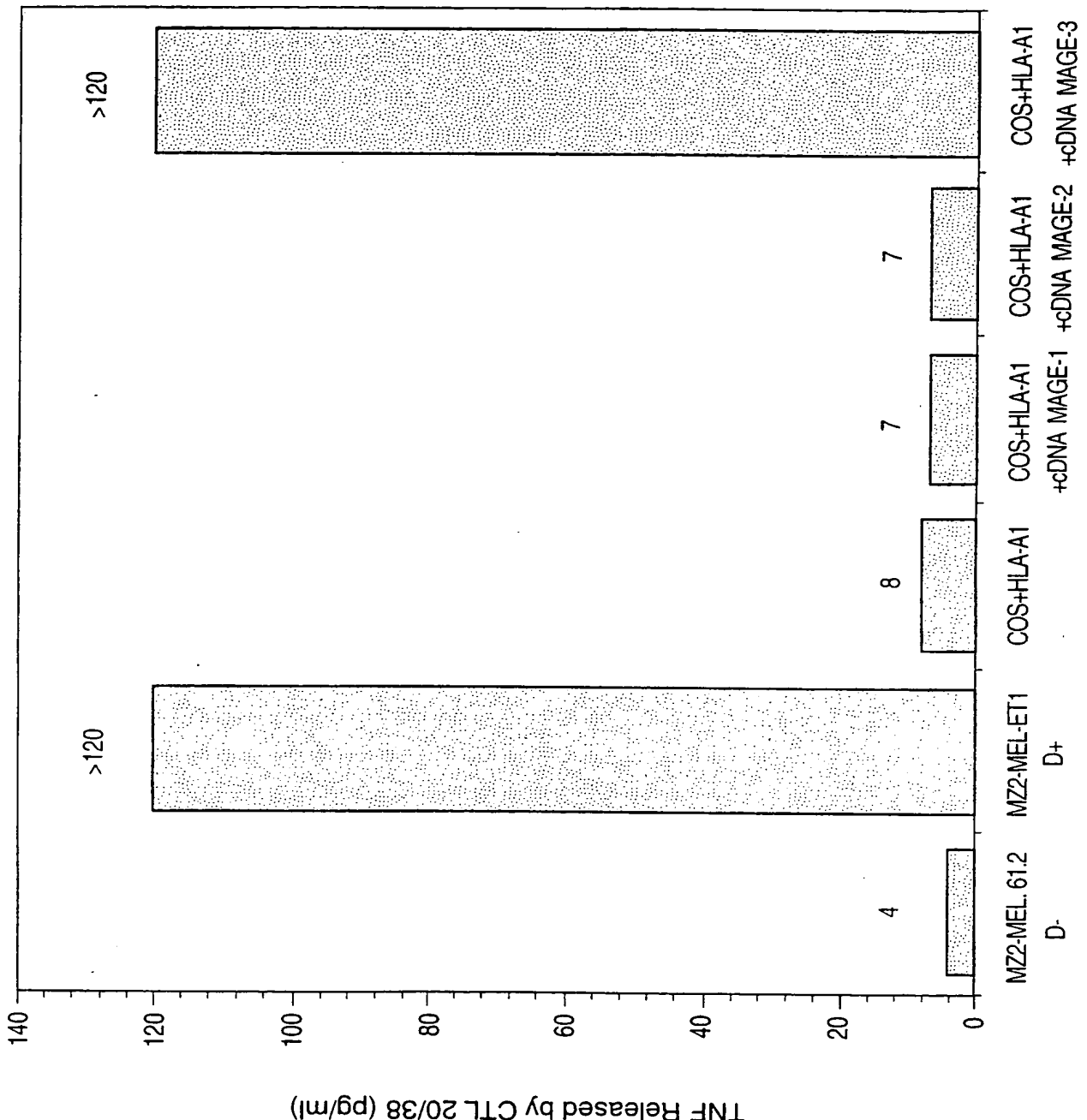

FIG. 16

BNSDOCID: <WO 9523874A1>

FIG. 17

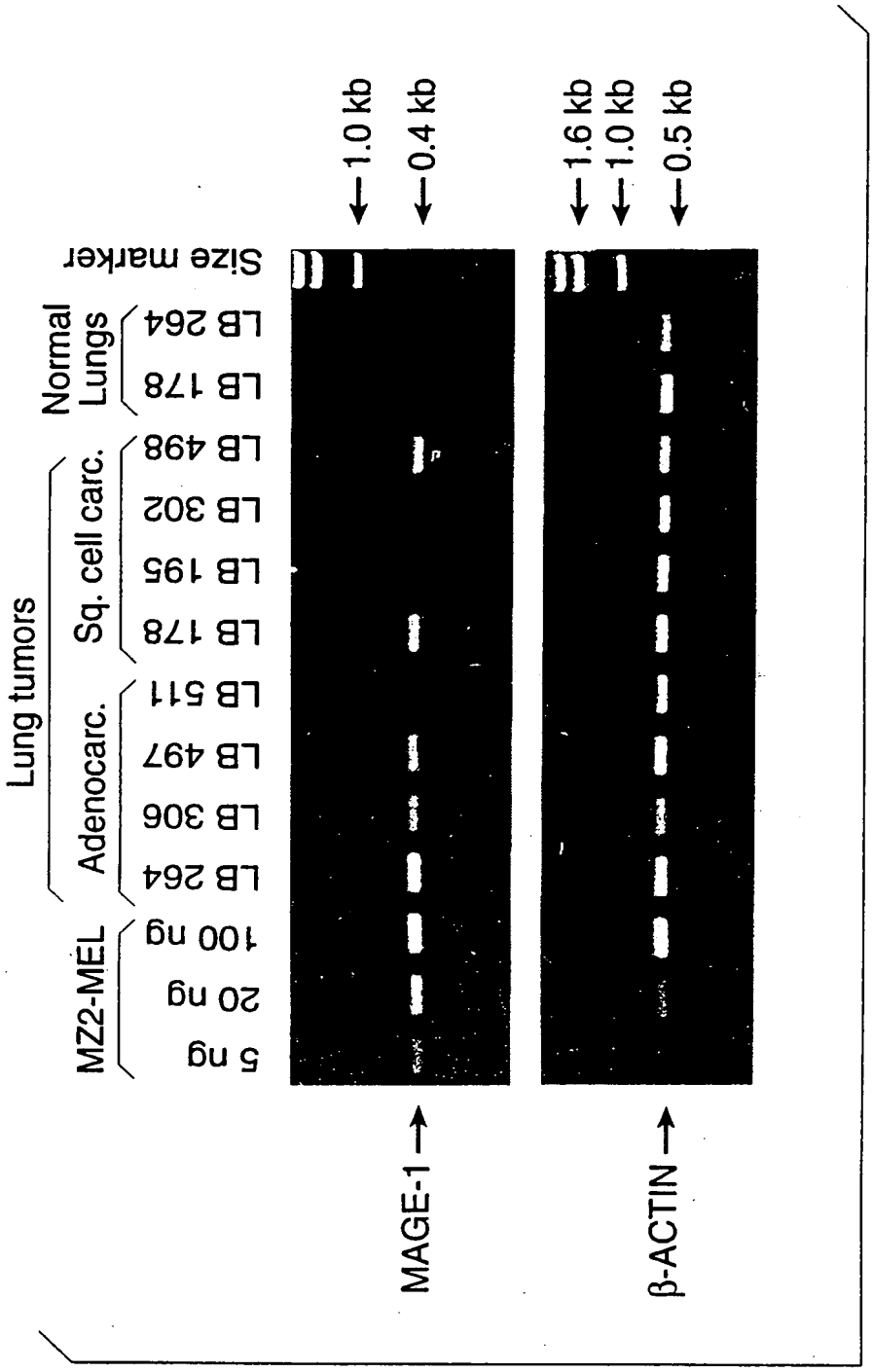

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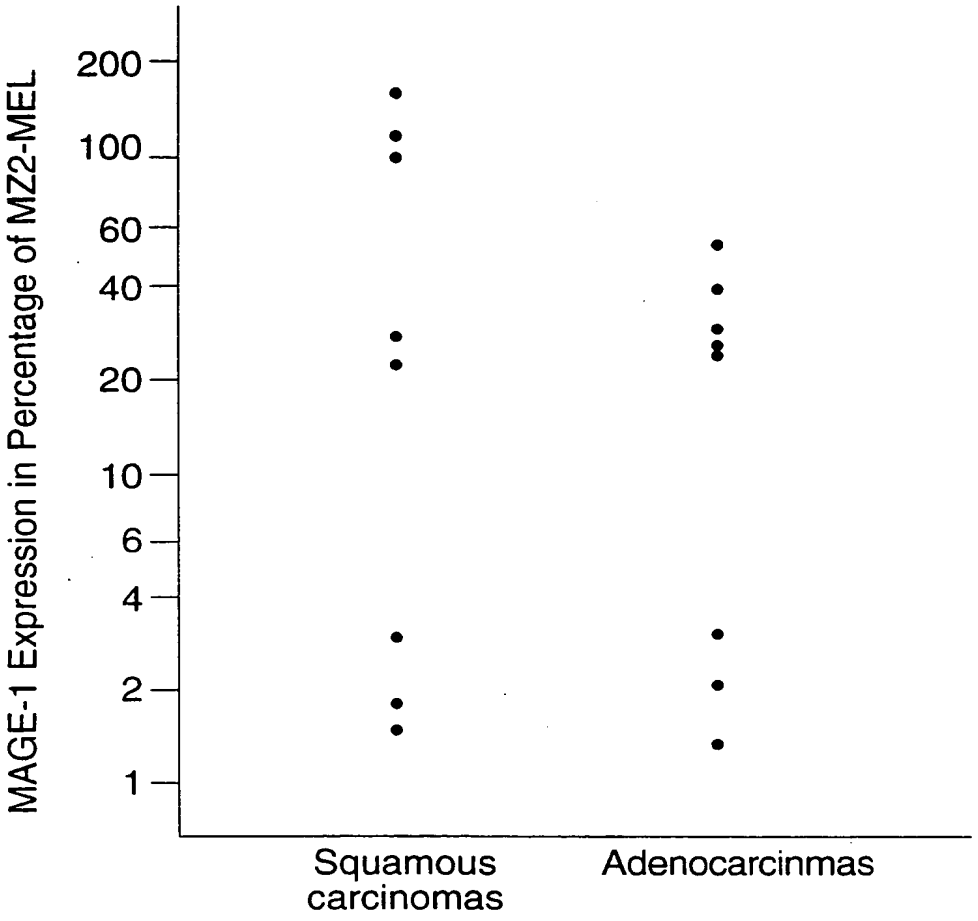
FIG. 18



BNSDOCID: <WO 9523874A1>

FIG. 19

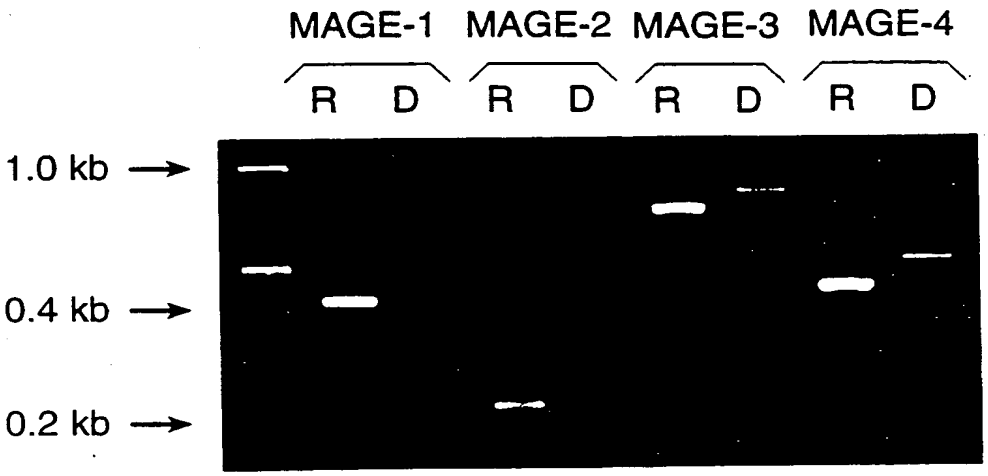


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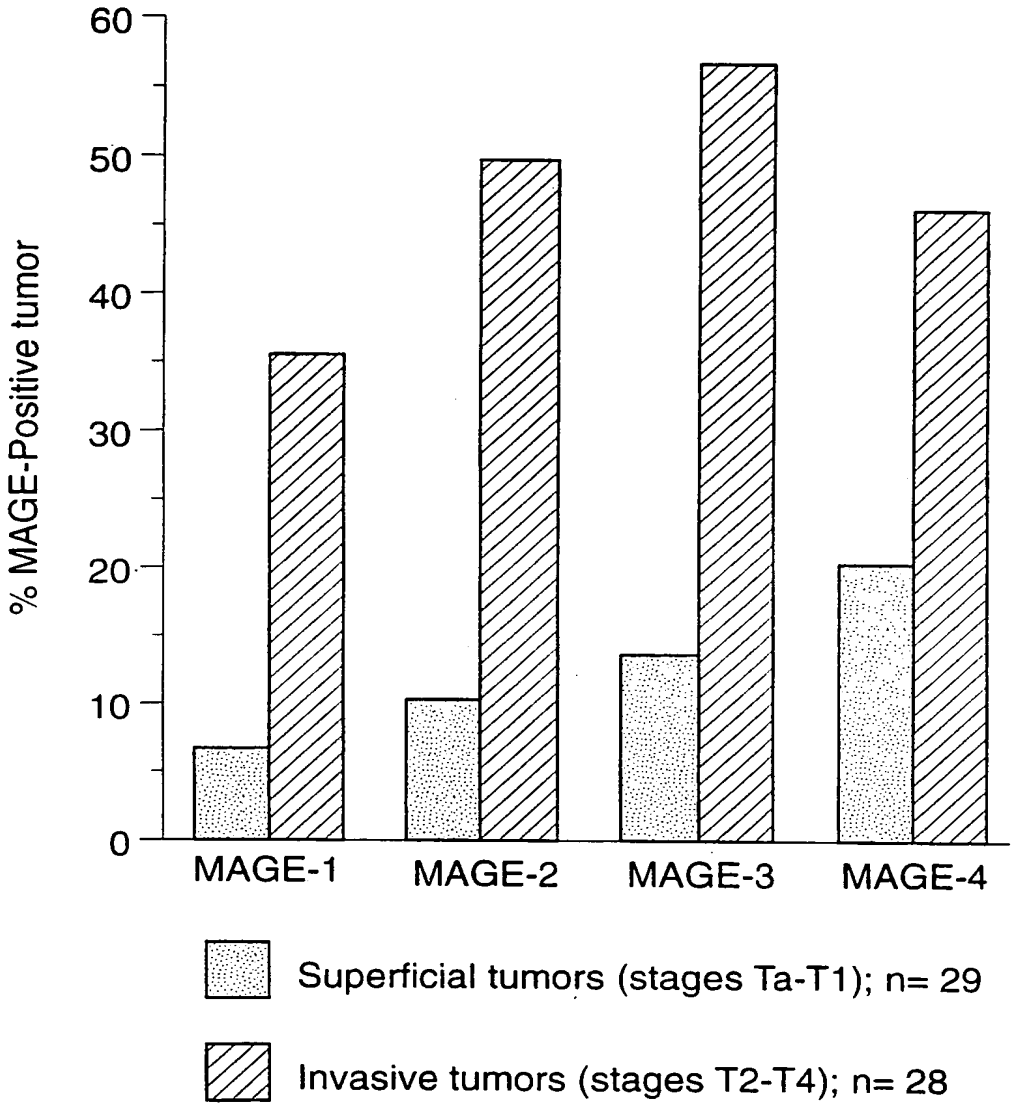
FIG. 20



BNSDOCID: <WO 9523874A1>

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/02203

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C12Q 1/68
US CL :435/6
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS, BIOSIS, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	IMMUNOGENETICS, VOLUME 39, ISSUED 1994, SMET ET AL. "SEQUENCE AND EXPRESSION PATTERN OF HUMAN MAGE2 GENE", PAGES 121-129, SEE ENTIRE DOCUMENT.	1-10
Y	INTERNATIONAL JOURNAL OF CANCER, ISSUED 1994, WEYNANTS ET AL, "EXPRESSION OF MAGE GENES BY NON-SMALL-CELL LUNG CARCINOMAS", PAGES 826-829, SEE ENTIRE DOCUMENT.	1-10
Y	WO, A, 92/20356 (BOON ET AL) 26 NOVEMBER 1992, SEE ENTIRE DOCUMENT.	1-10
A,P	US, A, 5,342,774 (BOON ET AL), 30 AUGUST 1994, SEE ENTIRE DOCUMENT.	1-10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 01 MAY 1995	Date of mailing of the international search report 24MAY1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer EGGERTON CAMPBELL Telephone No. (703) 308-0196

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